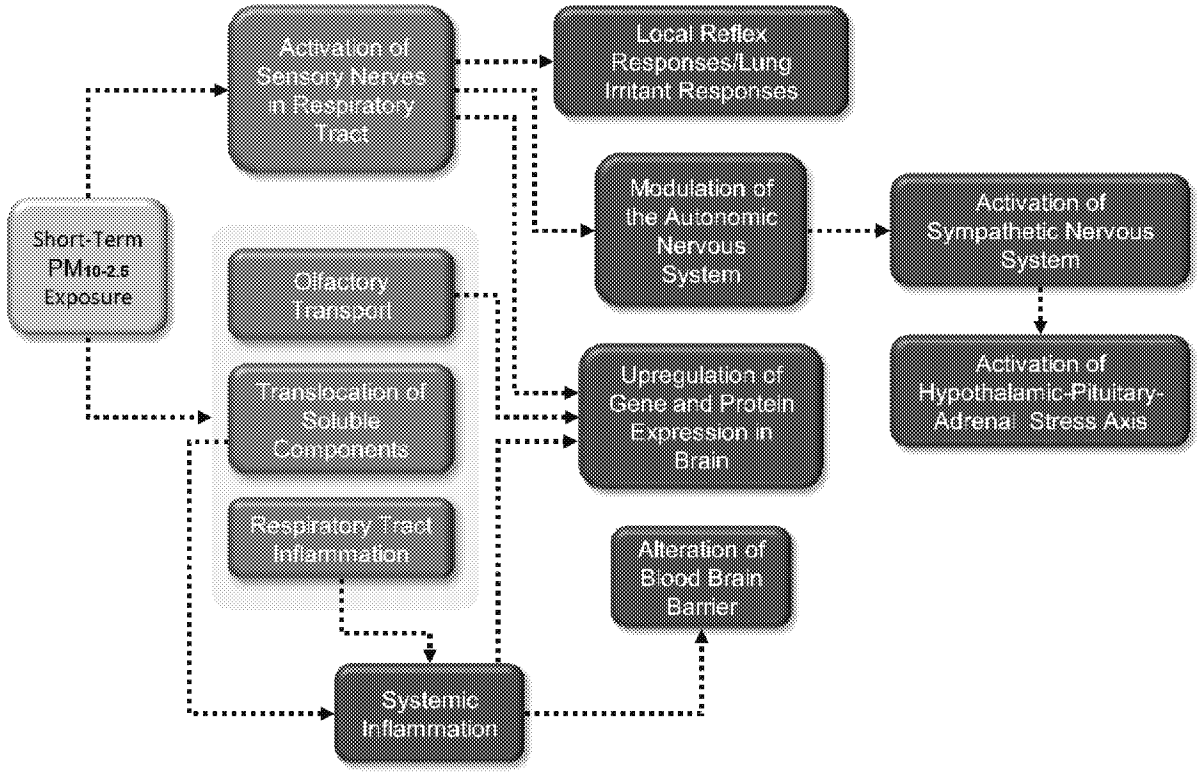
8.3.1 Biological Plausibility

This section describes biological pathways that potentially underlie nervous system effects resulting from short-term exposure to $PM_{10-2.5}$. Figure 8-8 graphically depicts the proposed pathways as a continuum of upstream events, connected by arrows, that may lead to downstream events observed in epidemiologic studies. This discussion of "how" short-term exposure to $PM_{10-2.5}$ may lead to nervous system effects contributes to an understanding of the biological plausibility of epidemiologic results evaluated later in Section 8.3.

Once $PM_{10-2.5}$ deposits in the respiratory tract, it may be retained, cleared, or solubilized (see Chapter 4). $PM_{10-2.5}$ and its soluble components may interact with cells in the respiratory tract, such as epithelial cells, inflammatory cells, and sensory nerve cells. One way in which this may occur is through reduction-oxidative (redox) reactions. As discussed in Section 2.3.3, PM may generate ROS and this capacity is termed "oxidative potential". Furthermore, cells in the respiratory tract may respond to the presence of PM by generating ROS. Further discussion of these redox reactions, which may contribute to oxidative stress, is found in Section 5.1.1 of the 2009 PM ISA (U.S. EPA, 2009). In addition, poorly soluble particles may translocate to the interstitial space beneath the respiratory epithelium and accumulate in the lymph nodes (see CHAPTER 4). Immune system responses due to the presence of particles in the interstitial space may contribute to health effects. Inflammatory mediators may diffuse from the respiratory tract into the systemic circulation and lead to inflammation in extrapulmonary compartments (Section 6.3.1). Although $PM_{10-2.5}$ is mostly insoluble, it may contain some soluble components such as endotoxin and metals. Soluble components of $PM_{10-2.5}$ may translocate into the systemic circulation and contribute to inflammatory or other processes in extrapulmonary compartments. A fraction of $PM_{10-2.5}$ may deposit on the olfactory epithelium. Soluble components of $PM_{10-2.5}$ may be transported via the olfactory nerve to the olfactory bulb of the brain. The extent to which translocation into the systemic circulation or transport to the olfactory bulb occurs is currently uncertain. For further discussion of translocation and olfactory transport, see CHAPTER 4.



Note: The boxes above represent the effects for which there is experimental or epidemiologic evidence, and the dotted arrows indicate a proposed relationship between those effects. Progression of effects is depicted from left to right and color-coded (gray, exposure; green, initial event; blue, intermediate event; orange, apical event). Here, apical events generally reflect results of epidemiologic studies, which often observe effects at the population level. Epidemiologic evidence may also contribute to upstream boxes. When there are gaps in the evidence, there are complementary gaps in the figure and the accompanying text below.

Figure 8-8 Potential biological pathways for nervous system effects following short-term $PM_{10-2.5}$ exposure.

Evidence that short-term exposure to $PM_{10-2.5}$ may affect the nervous system generally informs one pathway that begins with activation of sensory nerves in the respiratory tract. This can trigger local reflex responses and transmit signals to regions of the central nervous system that regulate autonomic outflow. Altered autonomic tone may result in effects in other organs (Figure 8-8). Decrements in lung function seen immediately after a 4-hour exposure to $PM_{10-2.5}$ in an animal toxicological study by Amatullah et al. (2012) indicates that activation of sensory nerves in the respiratory tract may have triggered a reflex response in the lung or that modulation of the ANS may have contributed to the observed effects (Section 5.3.6.3). In addition, evidence from a controlled human exposure study supports a link between short-term $PM_{10-2.5}$ exposure and activation of the HPA stress axis (Liu et al., 2017). In this way, the ANS may mediate systemic responses due to exposure to $PM_{10-2.5}$. Currently there are no epidemiologic studies evaluating the relationship between short-term exposure to $PM_{10-2.5}$ and nervous system effects.

An animal toxicological study found upregulation of gene and protein expression in the brain following short-term exposure to $PM_{10-2.5}$ (Ljubimova et al., 2013). Whether this response was due to altered autonomic tone or to systemic inflammation or olfactory transport is uncertain. This study was conducted in rodents, which are obligatory nasal breathers (as opposed to humans who are oro-nasal breathers). Deposition of $PM_{10-2.5}$ in the tracheobronchial or pulmonary regions of the lung of rodents is expected to be minimal. An effect seen in the brain of rodents indicates that $PM_{10-2.5}$, which deposited in the nose, may have activated sensory nerves in the nose. It is also possible that soluble components may have translocated into the systemic circulation or have been transported from the olfactory epithelium in the nose to the olfactory bulb in the brain via the axons of olfactory sensory neurons. Responses seen in the controlled human exposure study by Liu et al. (2017), which also found evidence linking exposure to $PM_{10-2.5}$ to altered blood brain barrier function, may reflect different patterns of deposition in oro-nasal breathers.

Summary of Biological Plausibility

As described here, there is one proposed pathway by which short-term exposure to $PM_{10-2.5}$ may lead to nervous system effects. Stimulation of receptors on sensory nerves, possibly in the nose, may trigger local reflex responses or transmit signals to the regions of the central nervous system that regulate autonomic outflow, resulting in activation of the SNS and the HPA stress axis. Experimental studies in animals and humans contribute all the evidence of upstream and downstream events. This proposed pathway will be used to inform a causality determination, which is discussed later in the chapter (Section 8.3.4).

8.3.2 Activation of the Sympathetic Nervous System and the Hypothalamic-Pituitary-Adrenal (HPA)Stress Axis

A controlled human exposure study examined the effects of a 130 minute exposure to $PM_{10-2.5}$ CAPs on urinary and blood biomarkers associated with neural effects (Liu et al., 2017). Associations between exposure to $PM_{10-2.5}$ CAPs and decreases in biomarkers related to blood brain barrier integrity, including blood S100 calcium-binding protein B and neuron-specific enolase, were observed at 21 hours post-exposure ($p < 0.1$). In addition, exposure to $PM_{10-2.5}$ CAPs was associated with increases in stress-related markers such as urinary vanillylmandelic acid and cortisol at 21 hours post-exposure ($p < 0.05$) and decreases in blood cortisol at 1 and 21 hours post-exposure ($p < 0.05$). Since vanillylmandelic acid is the primary metabolite resulting from breakdown of the stress-related hormones epinephrine and norepinephrine, its presence in urine indicates that exposure to $PM_{10-2.5}$ CAPs led to secretion of epinephrine and/or norepinephrine into the blood by the adrenal medulla subsequent to activation of the HPA stress axis. Increased levels of urinary cortisol, which is secreted into the blood by the adrenal cortex, also indicates that exposure to $PM_{10-2.5}$ CAPs led to activation of the HPA stress axis (Table 8-21).

Table 8-21 Study-specific details from a controlled human exposure study of short-term exposure to $PM_{10-2.5}$ and activation of the sympathetic nervous system (SNS)/hypothalamic-pituitary-adrenal (HPA) stress axis.

Study/Study Population	Pollutant	Exposure Details	Endpoints Examined
Liu et al. (2017) Species: Human Health status: Healthy nonsmokers Sex: 29 females, 26 males Age: 18–60 yr Study design: Single-blind randomized cross-over trial Single-blind randomized cross-over trial	CAPs from Toronto, ON Particle sizes: 2.5–10 μm Control: HEPA filtered ambient air or HEPA-filtered medical air (ultrafine study)	Route: Face mask inhalation Dose/concentration: 212.9 ± 52.0 $\mu g/m^3$ Duration of exposure: 130 min Time to analysis: 1 and 21 h	Urinary and blood markers of neural effects

CAPs = concentrated ambient particles; HEPA = high efficiency particulate absorber.

8.3.3 Brain Inflammation and Oxidative Stress

An animal toxicological study examined changes in global gene expression in the brain, as well as expression of Arc and Rac genes and their protein products, in Fischer 344 rats exposed to $PM_{10-2.5}$ CAPs in Riverside, CA for 2 weeks (Ljubimova et al., 2013). No changes in global gene expression were found. However, increased Arc gene expression ($p < 0.05$) and increased Arc immunostaining were observed. In contrast, exposure to $PM_{2.5}$ CAPs and UFP CAPs had no effects on these genes or their protein products (Table 8-22).

Table 8-22 Study-specific details from an animal toxicological study of short-term exposure to $PM_{10-2.5}$ and brain inflammation and oxidative stress.

Study/Study Population	Pollutant	Exposure Details	Endpoints Examined
Ljubimova et al. (2013) Species: Rat Sex: Male Strain: Fisher 344 Age/Weight: 3–7 weeks	CAPs from Riverside, CA (summer) Particle size 3,000 nm Control: Filtered air	Route: Whole body inhalation Dose/Concentration: 58 ± 7 μg/m³ Duration: 5 h/day, 4 days duration: 5 h/day, 4 days/week for 0.5 mo	Brain tissue—Immunohistochemistry Gene expression—mRNA

CAPs = concentrated ambient particles.

8.3.4 Summary and Causality Determination

There were no studies of the effect of $PM_{10-2.5}$ on the nervous system effects in adults or children reviewed in the 2009 PM ISA. The evidence characterizing the relationship between short-term exposure to $PM_{10-2.5}$ and effects on the nervous system is detailed below (Table 8-23), using the framework for causality determination described in the Preamble to the ISAs (U.S. EPA, 2015). The evidence base consists of a limited number of experimental studies without supporting epidemiologic studies. The toxicological study examined the potential for inhalation of $PM_{10-2.5}$ to affect the nervous system and found altered gene expression in the brain (Ljubimova et al., 2013). The controlled human exposure study indicated activation of the HPA stress axis in relation to short-term exposure to $PM_{10-2.5}$(Liu et al., 2017). **Overall, the evidence is inadequate to infer the presence or absence of a causal relationship between short-term $PM_{10-2.5}$ exposure and nervous system effects.**

Table 8-23 Summary of evidence that is inadequate to infer the presence or absence of a causal relationship between short-term $PM_{10-2.5}$ exposure and nervous system effects.

Rationale for Causality Determination[a]	Key Evidence[b]	Key References[b]	$PM_{2.5}$ Concentrations Associated with Effects[c]
Limited controlled human exposure study evidence	Changes in levels of metabolite of epinephrine/epinephrine and cortisol in urine indicate HPA stress axis activation	Liu et al. (2017)	212.9 µg/m^3
Lack of epidemiologic evidence	No studies of the association between short-term exposure to PM10-2.5 and nervous system effects reviewed		
Limited biological plausibility	Limited toxicological evidence of altered gene expression in brain	Ljubimova et al. (2013)	58 µg/m^3

[a]Based on aspects considered in judgments of causality and weight of evidence in causal framework in Table I and Table II of the Preamble.

[b]Describes the key evidence and references, supporting or contradicting, contributing most heavily to causality determination and, where applicable, to uncertainties or inconsistencies. References to earlier sections indicate where full body of evidence is characterized.

[c]Describes the $PM_{2.5}$ concentrations with which the evidence is substantiated (for experimental studies, ≤2 mg/m^3).

HPA = hypothalamic-pituitary-adrenal; SNS = sympathetic nervous system.

8.4 Long-term $PM_{10-2.5}$ Exposure and Nervous System Effects

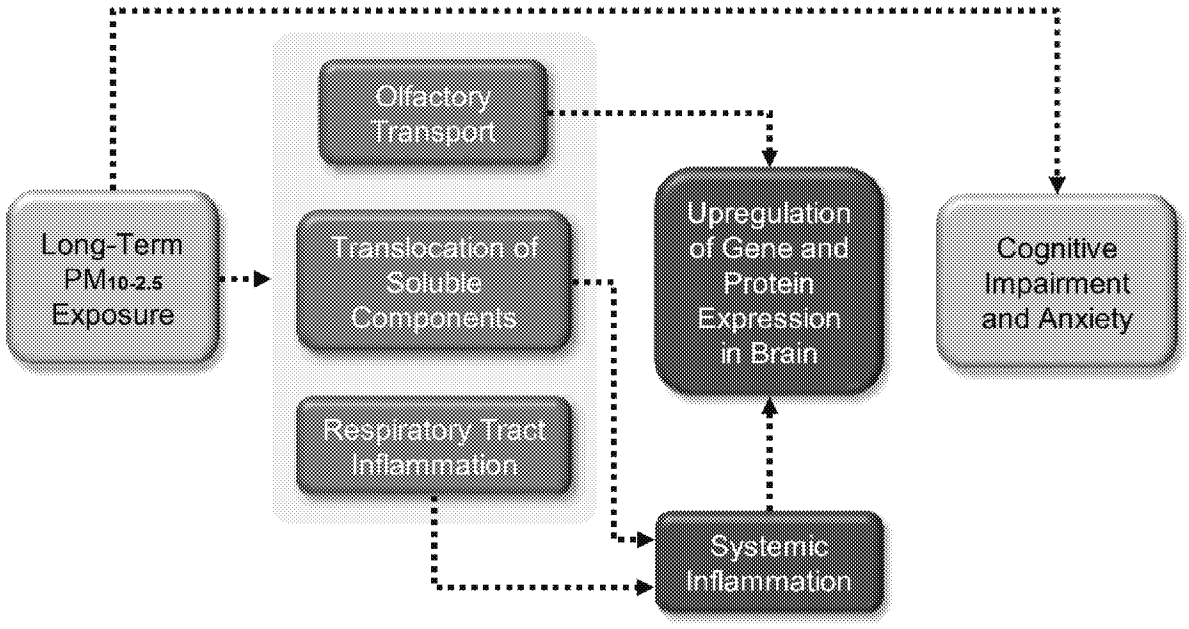
The previous ISA did not report any studies of nervous system effects as a result of long-term exposure to $PM_{10-2.5}$. There are some recent studies available for review. The discussion opens with a discussion of biological plausibility (Section 8.1.1) that provides background for the subsequent sections in which groups of related endpoints are presented in the context of relevant disease pathways. These outcome groupings are brain inflammation and oxidative stress (Section 8.4.2), cognitive and behavioral effects in adults (Section 8.4.3), and neurodevelopmental effects (Section 8.4.4). Finally, the collective body of evidence is integrated[74] across and within scientific disciplines, and the rationale for the causality determination is outlined in Section 8.1.6.

[74] As detailed in the Preface, risk estimates are for a 5 µg/m3 increase in annual PM10−2.5 concentrations unless otherwise noted.

8.4.1 Biological Plausibility

This section describes biological events that potentially underlie nervous system effects resulting from long-term exposure to $PM_{10-2.5}$. Figure 8-9 graphically depicts the continuum of upstream events, connected by arrows, that may lead to downstream events observed in epidemiologic studies. This discussion of "how" long-term exposure to $PM_{10-2.5}$ may lead to nervous system effects contributes to an understanding of the biological plausibility of epidemiologic results evaluated later in Section 8.4.

Once $PM_{10-2.5}$ deposits in the respiratory tract, it may be retained, cleared, or solubilized (see Chapter 4). $PM_{10-2.5}$ and its soluble components may interact with cells in the respiratory tract, such as epithelial cells, inflammatory cells, and sensory nerve cells. One way in which this may occur is through reduction-oxidative (redox) reactions. As discussed in Section 2.3.3, PM may generate ROS and this capacity is termed "oxidative potential". Furthermore, cells in the respiratory tract may respond to the presence of PM by generating ROS. Further discussion of these redox reactions, which may contribute to oxidative stress, is found in Section 5.1.1 of the 2009 PM ISA (U.S. EPA, 2009). In addition, poorly soluble particles may translocate to the interstitial space beneath the respiratory epithelium and accumulate in the lymph nodes (see Chapter 4). Immune system responses due to the presence of particles in the interstitial space may contribute to health effects. Inflammatory mediators may diffuse from the respiratory tract into the systemic circulation and lead to inflammation in extrapulmonary compartments (Section 6.4.1). Although $PM_{10-2.5}$ is mostly insoluble, it may contain some soluble components such as endotoxin and metals. Soluble components of $PM_{10-2.5}$ may translocate into the systemic circulation and contribute to inflammatory or other processes in extrapulmonary compartments. A fraction of $PM_{10-2.5}$ may deposit on the olfactory epithelium. Soluble components of $PM_{10-2.5}$ may be transported via the olfactory nerve to the olfactory bulb of the brain. The extent to which translocation into the systemic circulation or transport to the olfactory bulb occurs is currently uncertain. For further discussion of translocation and olfactory transport, see Chapter 4.



Note: The boxes above represent the effects for which there is experimental or epidemiologic evidence, and the dotted arrows indicate a proposed relationship between those effects. Progression of effects is depicted from left to right and color-coded (gray, exposure; green, initial event; blue, intermediate event; orange, apical event). Here, apical events generally reflect results of epidemiologic studies, which often observe effects at the population level. Epidemiologic evidence may also contribute to upstream boxes. When there are gaps in the evidence, there are complementary gaps in the figure and the accompanying text below.

Figure 8-9 Potential biological pathways for nervous system effects following long-term $PM_{10-2.5}$ exposure.

Evidence that long-term exposure to $PM_{10-2.5}$ may affect the nervous system is very sparse (Figure 8-9). Unlike the case for short-term exposure to $PM_{10-2.5}$, there is a lack of evidence that long-term $PM_{10-2.5}$ exposure results in activation of sensory nerves in the respiratory tract. An animal toxicological study found upregulation of gene and protein expression in the brain following long-term exposure to $PM_{10-2.5}$ (Ljubimova et al., 2013). Whether this response occurred secondarily to systemic inflammation or olfactory transport is uncertain. This study was conducted in rodents, which are obligatory nasal breathers. Deposition of $PM_{10-2.5}$ in the tracheobronchial or pulmonary regions of the lung of rodents is expected to be minimal. An effect seen in the brain of rodents indicates that soluble components of $PM_{10-2.5}$ that was deposited in the nose, may have translocated into the systemic circulation or have been transported from the olfactory epithelium in the nose to the olfactory bulb in the brain via the axons of olfactory sensory neurons. Currently, epidemiologic evidence is limited to studies linking long-term $PM_{10-2.5}$ exposure to impaired cognition and to anxiety. The evidence of upstream events is insufficient to support a pathway that could be used to inform a causality determination, which is discussed later in the chapter (Section 8.4.5).

8.4.2 Brain Inflammation and Oxidative Stress

The previous ISA did not report any studies of nervous system effects as a result of long-term exposure to $PM_{10-2.5}$. The body of evidence continues to be limited (Table 8-24) and consists of an animal toxicological study that examined changes in global gene expression in the brain, as well as expression of Arc and Rac genes and their protein products in Fischer 344 rats exposed to $PM_{10-2.5}$ CAPs from Riverside, CA for 10 months (Ljubimova et al., 2013). No changes in global gene expression were found. However, exposure to $PM_{10-2.5}$ CAPs upregulated Arc at 1 and 3 months and downregulated Arc at 10 months ($p < 0.05$). Expression of Rac1 was increased following 10 months of exposure to $PM_{10-2.5}$ CAPs ($p < 0.01$). Immunostaining for Arc and Rac1 protein following 10-month exposure to $PM_{10-2.5}$ CAPs demonstrated no increases. In contrast, exposure to $PM_{2.5}$ CAPs and UFP CAPs had no effects on these genes or their protein products.

Table 8-24 Study-specific details from an animal toxicological study of long-term exposure to $PM_{10-2.5}$ and brain inflammation and oxidative stress.

Study/Study Population	Pollutant	Exposure Details	Endpoints Examined
Ljubimova et al. (2013) Species: Rat Sex: Male Strain: Fisher 344 Age/Weight: 3–7 weeks	CAPs from Riverside, CA (summer) Particle size: 3,000 nm Control: Filtered air	Route: Whole body inhalation Dose/Concentration: 58 ± 7 μg/m^3 Duration: 5 h/day, 4 days/week for 1, 3, and 10 mo	Brain tissue—Immunohistochemistry Gene expression—mRNA

CAPs = concentrated ambient particles.

8.4.3 Cognitive and Behavioral Effects in Adults

There were no studies examining the association of $PM_{10-2.5}$ with nervous system effects in adults reviewed in the 2009 PM ISA. Although the evidence remains limited, a small number of studies indicate the potential for long-term exposure to $PM_{10-2.5}$ to affect the nervous system of adults (Table 8-24).

The evidence relevant to the effect of long term exposure to $PM_{10-2.5}$ is limited to a small number of epidemiologic studies. Among women enrolled in the NHS, Weuve et al. (2012) reported faster cognitive decline in association with increased $PM_{10-2.5}$ exposure. The magnitude of the change between successive 2–year outcome measurement [−0.018 (95% CI: −0.035, −0.002)] persisted after adjustment for potential confounders (i.e., age, education, physical activity, alcohol consumption.). The correlation between long-term $PM_{2.5}$ and $PM_{10-2.5}$ concentrations was low (spearman correlation 0.20). Notably, the

association with cognitive decline remained after additional adjustment for cardiovascular risk factors and SES. In another analysis of the NHS cohort, Power et al. (2015) observed a small positive association between high anxiety and the annual average concentration of $PM_{10-2.5}$ [OR: 1.03 (95% CI: 0.99, 1.06)]. Associations generally weakened with shorter averaging times in this study. A large imprecise association between long-term exposure to $PM_{10-2.5}$ and mild cognitive impairment (MCI) was observed in a cross-sectional analysis of the HNR study [OR: 1.69 (95% CI: 0.90, 3.18)] (Tzivian et al., 2016). The association was stronger when MCI was defined to identify cases of amnestic MCI (i.e., objective impairment in at least one memory domain).

8.4.4 Neurodevelopmental Effects

There were no studies examining the association of $PM_{10-2.5}$ with neurodevelopmental effects reviewed in the 2009 PM ISA. The limited number of recently available studies do not provide strong evidence of an association (Table 8-25).

In a prospective study of children born in Rome and followed through age 7 when the WISC-III was administered to measure cognitive function, Porta et al. (2015) reported small (relative to the size of the confidence interval), imprecise associations between $PM_{10-2.5}$ and decrement on FSIQ in fully adjusted models [−1.10 (95% CI: −2.80, 0.50)]. A slightly larger decrease was observed on the Performance IQ subtest. Raz et al. (2015) reported little evidence association between PM10-2.5 and ASD in a case control study nested within the NHS cohort [e.g. OR: 1.07 (95%CI: 0.92, 1.24) third trimester exposure, which was the strongest association]. Findings from the Guxens et al. (2014) analysis of six European cohorts did not support a strong association with reduced general cognition or global psychomotor development [Coefficient: 0.59 (95%CI: −0.99, 2.17) and Coefficient: 0.42 (95% CI: −1.28, 0.45), respectively].

Table 8-25 Characteristics of the studies examining the association of long-term $PM_{10-2.5}$ exposures with cognitive function, behavioral and neurodevelopmental effects.

Study Location/Years	Study Population	Exposure Assessment	Concentration µg/m^3	Outcome	Copollutant Examination
†(Weuve et al., 2012) 11 US states Longitudinal Cohort $PM_{10-2.5}$: 1988–2007	NHS Women ≥70 yr N = 19,409	1 mo, 1 yr, 2 yr, 5 yr avg prior to baseline assessment. spatio-temporal, at residence (pre–1999 $PM_{2.5}$ estimated from PM_{10} ratio) Yanosky et al. (2008)	5 yr avg: 8.5	TICS Global score	Correlations (r): $PM_{2.5}$ r = 0.1–0.22 depending on metric Copollutant model: NR
†Power et al. (2015) Longitudinal cohort $PM_{10-2.5}$: 1988–2004 Outcome: 2004	NHS N = 7,1271 Mean age 70 yr	Multi-yr, annual avg, 1 mo, 3 mo and 6 mo prior to outcome, spatio-temporal, at residence (pre–1999 $PM_{2.5}$ estimated from PM_{10} ratio) Yanosky et al. (2008)	Mean (SD): 1 mo 7.27 (4.84); 3 mo 7.58 (4.72); 6 mo 6.99 (4.39); 12 mo 7.08 (4.25); 1988–2003 = 9.0 (4.1)	Crown-Crisp phobic anxiety scale score ≥6 (prevalent)	Correlations (r); $PM_{2.5}$ r=0.24 multi-yr avg Copollutant model: NR
†Tzivian et al. (2016) German Ruhr area Cross-sectional $PM_{10-2.5}$: 2008–2009 Outcome: 2006/2008	HNR study N = 4,086 50–80 yr	Annual avg at residential address, LUR, R2 for modelled and measured $PM_{10-2.5}$ = 0.66	Mean 18.39 (SD: 1.05) IQR: 1.4	MCI (Petersen/International Working group on MCI criteria) (Petersen, 2004)	Correlations (r): NR Copollutant models: NR
†(Porta et al., 2015) Rome, Italy Prospective Cohort $PM_{10-2.5}$: 2010–2011 Outcome: 2010–2011	GASPII Children 7 yr N = 474	Avg during pregnancy and from birth through age 7 at residence, LUR, C-V R2 = 0.57	Mean 19.5	WISC III	Correlations (r): NR Copollutant models: NR

Study Location/Years	Study Population	Exposure Assessment	Concentration µg/m^3	Outcome	Copollutant Examination
†Raz et al. (2015) 14 States, U.S. Nested case-control Births: 1990-2002	NHS n = 245 cases, n = 1522 noncases 1-3 yr	Spatiotemporal model to estimate concentration before, during, after pregnancy, at residence, difference method for $PM_{10-2.5}$ Yanosky et al. (2008)	Mean 9.9	ASD	Correlations (r): NR Copollutant models: NR
†Guxens et al. (2014) Six European cohorts 1997-2008 $PM_{10-2.5}$: 2008-2011 (back extrapolated)	ESCAPE N = 9482, 1-6 yr	LUR to estimated exposure during pregnancy at residence at time of birth,	NR	Cognitive and psychomotor development (BSID, DDST, MCDI, MIDI, MSCA)	Correlations (r): dependent on the cohort Copollutant models: NR

ASD=autism spectrum disorder; BSID=Bayley Scales of Infant Development; DDST=Denver Developmental Screening Test II; GASPII = Italian Cohort of the Environmental Health Risk in European Birth Cohorts; HNRS = Heinz Nixdorf Recall Study; LUR = Land Use Regression; MCDI=McArthur Communicative Development Inventory; MIDI = Minnesota Infant Development Inventory; MSCA= McCarthy Scales of Children's Abilities; MCI = Mild Cognitive Impairment; NHS = Nurses' Health Study; TICS = Telephone interview for Cognitive Status; WISC = Wechsler Intelligence Scale for Children.

†Studies published since the 2009 PM ISA.

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8.4.5 Summary and Causality Determination

There were no studies of the effect of $PM_{10-2.5}$ on the nervous system effects included in the 2009 PM ISA. Several recent epidemiologic studies that report the association of long-term exposure to $PM_{10-2.5}$ with cognitive and behavioral effects in adults but not with neurodevelopmental effects in children, are available for review. The evidence characterizing the relationship between long-term exposure to $PM_{2.5}$ and effects on the nervous system is detailed below (Table 8-25), using the framework for causality determination described in the Preamble to the ISAs (U.S. EPA, 2015).

Although there is a limited number of studies overall, the evidence base includes well-conducted epidemiologic studies reporting associations with impaired cognition and anxiety in longitudinal analyses of women enrolled in the NHS (Power et al., 2015; Weuve et al., 2012). Studies of long-term exposure during pregnancy or childhood were not consistently associated with neurodevelopmental effects. There is uncertainty stemming from exposure assessment methods relying on the difference method to estimate $PM_{10-2.5}$ concentration (Sections 2.4.2) and related uncertainties due to the potentially uncharacterized spatial variation in $PM_{10-2.5}$ (Section 2.5 and Section 3.3.1.1). None of the available studies adjusted for copollutant exposures. An experimental animal study examined the potential for inhalation of $PM_{10-2.5}$ CAPs to affect the nervous system and found altered gene expression in the brain (Ljubimova et al., 2013). **Overall, the evidence is suggestive of, but not sufficient to infer, a causal relationship between long-term $PM_{10-2.5}$ exposure and nervous system effects.**

Table 8-26 Summary of evidence that is suggestive of, but not sufficient to infer, a causal relationship between long-term $PM_{10-2.5}$ exposure and nervous system effects.

Rationale for Causality Determination[a]	Key Evidence[b]	Key References[b]	$PM_{2.5}$ Concentrations Associated with Effects[c]
Cognitive and Behavioral Effects			
High quality epidemiologic study shows an association	Accelerated 2-yr decline in cognitive score (TICs) in longitudinal analysis women of NHS	Weuve et al. (2012)	8.5 µg/m^3
		Power et al. (2015)	7.08 µg/m^3
		Tzivian et al. (2016)	18.39 µg/m^3
	Associations with anxiety in NHS and MCI in the HNR study		
Uncertainty related to exposure measurement error	Epidemiologic studies use difference method to estimate exposure to $PM_{10-2.5}$	Section 2.4.2 Section 2.5 Section 3.3.1.1	

Table 8-26 (Continued): Summary of evidence that is suggestive of, but not sufficient to infer, a causal relationship between long-term exposure to $PM_{10-2.5}$ and nervous system effects.

Rationale for Causality Determination[a]	Key Evidence[b]	Key References[b]	$PM_{2.5}$ Concentrations Associated with Effects[c]
	Potentially uncharacterized spatial variation adds additional uncertainty		
Uncertainty related to the independent effect of PM10-2.5	No studies reported copollutant model results.		
Biological Plausibility	Limited toxicological evidence of altered gene expression in brain	Ljubimova et al. (2013)	58 µg/m^3

[a]Based on aspects considered in judgments of causality and weight of evidence in causal framework in Table I and Table II of the Preamble.

[b]Describes the key evidence and references, supporting or contradicting, contributing most heavily to causality determination and, where applicable, to uncertainties or inconsistencies. References to earlier sections indicate where full body of evidence is characterized.

[c]Describes the $PM_{2.5}$ concentrations with which the evidence is substantiated (for experimental studies, ≤2 mg/m^3).

†Studies published since the 2009 PM ISA.

8.5 Short-term UFP Exposure and Nervous System Effects

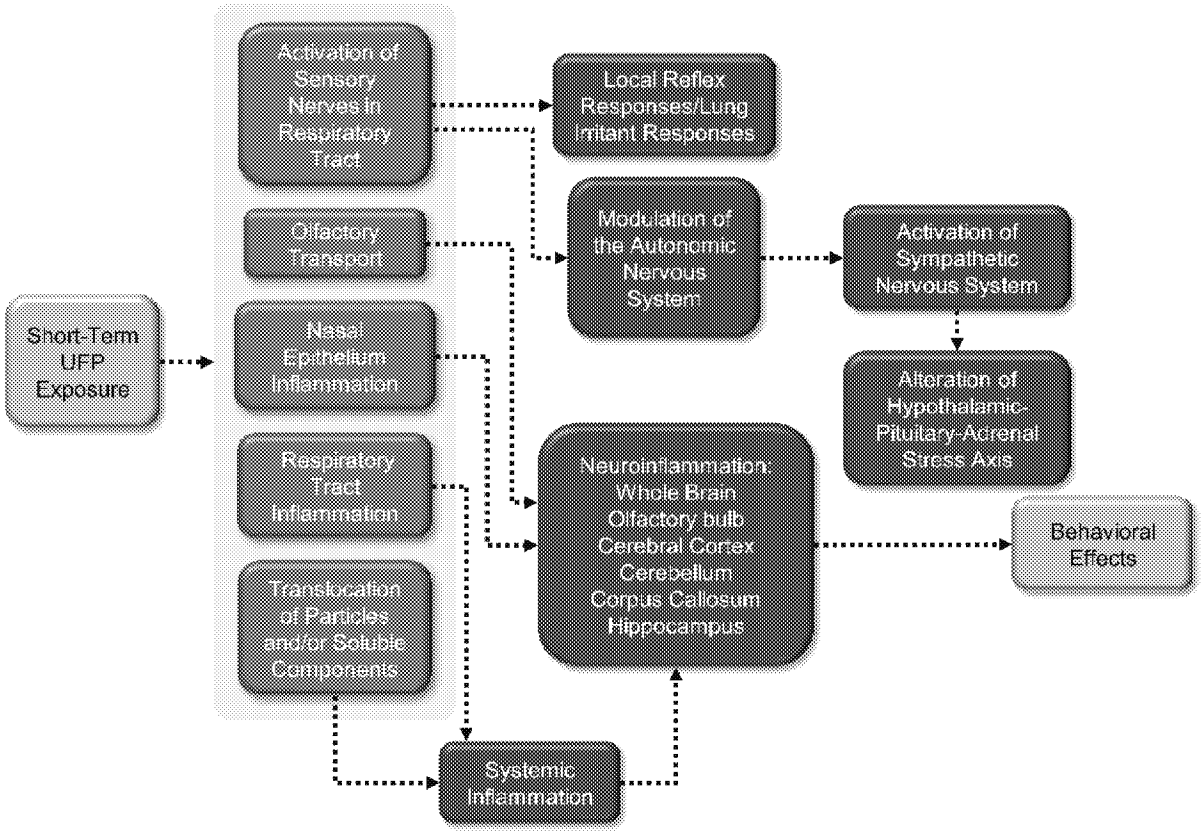
The previous ISA reported limited evidence of a relationship between exposure to ultrafine PM (UFP) and nervous system effects. An experimental study demonstrated that inhalation of UFP CAPs enhanced pro-inflammatory responses in the brains of mice that had been sensitized and challenged with ovalbumin (Campbell et al., 2005). Non-allergic mice were not tested. In addition, experimental studies in rodents previously found that inhaled laboratory-generated UFP can translocate from the olfactory epithelium to the olfactory bulb via the axons of olfactory sensory neurons (Elder et al., 2006; Oberdörster et al., 2004). Furthermore, magnetite UFP (10−150 nm), likely derived from combustion sources, have recently been found in frontal tissue from brains of humans (Maher et al., 2016). These findings suggest that ambient UFP may reach the brain via olfactory transport; however, other routes of translocation have not been ruled out (see Chapter 4).

The discussion of short-term UFP exposure and nervous system effects opens with a discussion of biological plausibility (Section 8.1.1) that provides background for the subsequent sections in which groups of related endpoints are presented in the context of relevant disease pathways. These outcome groupings are activation of the SNS and HPA stress axis (Section 8.5.2), brain inflammation and oxidative stress (Section 8.5.3), cognitive and behavioral effects in adults (Section 8.5.4). Finally, the collective body of evidence is integrated across and within scientific disciplines, and the rationale for the causality determination is outlined in Section 8.1.6.

8.5.1 Biological Plausibility

This section describes biological pathways that potentially underlie nervous system effects resulting from short-term exposure to UFP. Figure 8-10 graphically depicts the proposed pathways as a continuum of upstream events, connected by arrows, that may lead to downstream events observed in epidemiologic studies. This discussion of "how" short-term exposure to UFP may lead to nervous system effects contributes to an understanding of the biological plausibility of epidemiologic results evaluated later in Section 8.5.

Once UFP deposits in the respiratory tract, it may be retained, cleared, or solubilized (see Chapter 4). UFP and its soluble components may interact with cells in the respiratory tract, such as epithelial cells, inflammatory cells, and sensory nerve cells. One way in which this may occur is through reduction-oxidative (redox) reactions. As discussed in Section 2.3.3, PM may generate ROS and this capacity is termed "oxidative potential". Furthermore, cells in the respiratory tract may respond to the presence of PM by generating ROS. Further discussion of these redox reactions, which may contribute to oxidative stress, is found in Section 5.1.1 of the 2009 PM ISA (U.S. EPA, 2009). In addition, poorly soluble particles may translocate to the interstitial space beneath the respiratory epithelium and accumulate in the lymph nodes (see Chapter 4). Immune system responses due to the presence of particles in the interstitial space may contribute to health effects. Inflammatory mediators may diffuse from the respiratory tract into the systemic circulation and lead to inflammation in extrapulmonary compartments (Section 6.5.1). UFP and its soluble components may translocate into the systemic circulation and contribute to inflammatory or other processes in extrapulmonary compartments. A fraction of UFP may deposit on the olfactory epithelium. UFP and its soluble components may be transported via the olfactory nerve to the olfactory bulb of the brain. The extent to which translocation into the systemic circulation or transport to the olfactory bulb occurs is currently uncertain. For further discussion of translocation and olfactory transport, see Chapter 4.



Note: The boxes above represent the effects for which there is experimental or epidemiologic evidence, and the dotted arrows indicate a proposed relationship between those effects. Progression of effects is depicted from left to right and color-coded (gray, exposure; green, initial event; blue, intermediate event; orange, apical event). Here, apical events generally reflect results of epidemiologic studies, which often observe effects at the population level. Epidemiologic evidence may also contribute to upstream boxes. When there are gaps in the evidence, there are complementary gaps in the figure and the accompanying text below.

Figure 8-10 Potential biological pathways for nervous system effects following short-term UFP exposure.

Evidence that short-term exposure to UFP may affect the nervous system generally informs two different pathways (Figure 8-10). The first pathway begins with the activation of sensory nerves in the respiratory tract that can trigger local reflex responses and transmit signals to regions of the central nervous system that regulate autonomic outflow. The second pathway begins with pulmonary inflammation, leading to systemic inflammation and resulting in inflammation in the brain. Inflammation may lead to a worsening of neurodegenerative disease. Evidence for these pathways is described below.

Activation of Sensory Nerves and Modulation of the Autonomic Nervous System (ANS)

With regard to the first pathway, activation of sensory nerves in the respiratory tract may trigger local reflex responses in the lungs or modulate the ANS. Changes in lung function observed in controlled human exposure (Jr et al., 2008) and epidemiologic (McCreanor et al., 2007) (Mirabelli et al., 2015) studies potentially link short-term UFP exposure to the triggering of local reflex responses. However, inflammation (see below) may also play a role in lung function changes observed following short-term UFP exposure.

Evidence for changes in the HPA stress axis is provided by a controlled human exposure study that demonstrated an increase in a marker of the HPA stress axis in association with UFP exposure (Liu et al., 2017). Decreased levels of norepinephrine in the hypothalamus and decreased levels of serum glucocorticoids were observed in an animal toxicological study (Allen et al., 2014b) and indicate that UFP exposure may lead to other perturbations of the SNS and HPA stress axis.

Inflammation

With regard to the second pathway, deposition of UFP in the respiratory tract may lead to pulmonary inflammation (see Section 5.5.1) and to systemic inflammation (see Section 6.5.1), which in turn may lead to inflammation in the brain. Brain inflammation may be due to peripheral immune activation (Fonken et al., 2011) or to systemic circulation of UFP that results in particle uptake in the brain (Ljubimova et al., 2013). Inflammation in the brain may alternatively occur following olfactory transport of poorly soluble particles or their soluble components or to a neuroendocrine stress response resulting from activation of the HPA stress axis (Kodavanti, 2016).

Animal toxicological studies demonstrated neuroinflammation in several brain regions, including olfactory bulb, cerebral cortex, cerebellum, corpus callosum, and hippocampus following short-term UFP exposure (Cheng et al., 2016), (Allen et al., 2014b), (Tyler et al., 2016), (Campbell et al., 2005). Some responses were sex-specific (Allen et al., 2014b). Inflammation, oxidative stress, and apoptotic responses were also observed in nasal epithelium (Cheng et al., 2016). These changes preceded changes measured in olfactory bulb, cerebral cortex, and cerebellum in the same study. Evidence of these time-dependent and region-specific responses indicates that both olfactory transport and systemic inflammation may have played a role in responses to UFP exposure. In addition, paracrine signaling of inflammatory mediators between the nasal epithelium and proximal regions of the brain may have contributed to inflammation. In Tyler et al. (2016), inflammation in the brain occurred in the absence of pulmonary or systemic inflammation, pointing to a direct effect of UFP on the brain. Behavioral effects were found in conjunction with neuroinflammation in one study (Allen et al., 2013).

Summary of Biological Plausibility

As described here, there are two proposed pathways by which short-term exposure to UFP may lead to nervous system effects. The first pathway begins with activation of sensory nerves in the respiratory tract and may lead to triggering of lung reflex responses and modulation of the ANS resulting in increased activity of the SNS and stimulation of the HPA stress axis. In this way, the ANS may mediate systemic responses resulting from UFP exposure. The second proposed pathway begins with pulmonary/systemic inflammation or olfactory transport of UFP and may lead to pro-inflammatory effects in the brain and subsequently to behavioral effects. Animal toxicological and controlled human exposure studies provide the evidence for upstream and downstream events. There are no epidemiologic studies that evaluated the relationship between short-term exposure to UFP and nervous system effects. The proposed pathways will be used to inform a causality determination, which is discussed later in the chapter (Section 8.5.5).

8.5.2 Activation of the Sympathetic Nervous System and the Hypothalamic-Pituitary-Adrenal (HPA) Stress Axis

8.5.2.1 Controlled Human Exposure Study

A controlled human exposure study (Table 8-27) examined the effects of a 130 minute exposure to UFP CAPs on urinary and blood biomarkers associated with neural effects (Liu et al., 2017). An association between exposure to UFP CAPs and an increase in urinary vanillylmandelic acid, a stress-related biomarker, was observed at 1-hour post-exposure ($p < 0.1$). Vanillylmandelic acid is the primary metabolite resulting from the breakdown of the stress hormones epinephrine and norepinephrine. Its presence in urine indicates that exposure to UFP CAPs led to secretion of epinephrine and/or norepinephrine into the blood by the adrenal medulla subsequent to activation of the HPA stress axis.

Table 8-27 Study-specific details from a controlled human exposure study of short-term exposure to UFP and activation of the sympathetic nervous system (SNS) and hypothalamic-pituitary-adrenal (HPA) stress axis.

Study/Study Population	Pollutant	Exposure Details	Endpoints Examined
Liu et al. (2017) Species: Human Health status: Healthy nonsmokers Sex: 29 females, 26 male Age: 18–60 yr	CAPs from Toronto, ON Particle sizes: <0.3 µm	Route: Face mask inhalation Dose/concentration: 135.8 ± 67.2 µg/m^3 Particle number count 227,767 ± 63,902	Urinary and blood markers of neural effects

Study/Study Population	Pollutant	Exposure Details	Endpoints Examined
Study design: Single-blind randomized cross-over trial	Control: HEPA filtered ambient air or HEPA-filtered medical air (ultrafine study)	Duration of exposure: 130 min Time to analysis: 1 and 21 h	

CAPs = concentrated ambient particles; HEPA = high efficiency particulate absorber.

8.5.2.2 Animal Toxicological Study

Allen et al. (2014b) reported changes in neurotransmitters in adult mice exposed for 4 days to UFP CAPs beginning at PND 56 (Table 8-28). Brain tissue was analyzed at 9 months. Neurotransmitters were altered by exposure to CAPs in a sex- and brain region-specific manner. Most notably, exposure resulted in decreased norepinephrine in the hypothalamus of male mice and increased norepinephrine in the midbrain of female mice ($p < 0.05$). Allen et al. (2014b) also examined serum corticosterone levels in male and female mice exposed to UFP CAPS. Blood samples were collected at PND 60 and at about 6 months of age. At both time points, exposure decreased serum corticosterone levels in males ($p < 0.05$), but had no effect in females.

Table 8-28 Study-specific details from an animal toxicological study of short-term exposure to UFP and activation of the sympathetic nervous system (SNS) and hypothalamic-pituitary-adrenal (HPA) stress axis.

Study/Study Population	Pollutant	Exposure Details	Endpoints Examined
Allen et al. (2014b) Species: Mouse Sex: male and female Strain: C57BL/6J Age/Weight: Adult exposure at PND 56-60	CAPs collected in Rochester, NY from a "nearby highly trafficked roadway" using the Harvard University Concentrated Ambient Particle System Particle size: ≤100 nm Control: HEPA-filtered room air	Route: Whole body inhalation Dose/Concentration: 67.9 µg/m^3 Particle number: 180,000–200,000 particles/cm^3 Duration: 4 h/day, 4 days Time to analysis: 9 mo of age for brain tissue analysis PND 60 and 6 mo of age for blood collection	Brain tissue—Region specific levels of monoamines, amino acids Blood—corticosterone

CAPs = concentrated ambient particle; HEPA = high efficiency particulate absorber; PND = postnatal day.

8.5.3 Brain Inflammation and Oxidative Stress

Several animal toxicological studies provide evidence for brain inflammation and oxidative stress following short-term exposure to UFP (Table 8-29). Cheng et al. (2016) examined the effects of exposure to UFP on inflammatory and oxidative stress responses in olfactory epithelium, olfactory bulb, cerebral cortex, and cerebellum. Ambient UFP was collected near a freeway in Los Angeles, CA and re-aerosolized in order to expose C57BL/6J mice for 5, 20, and 45 hours over 3 weeks. Increases in oxidative stress markers, 4-hydroxy-2-nonenal and 3-nitrotyrosine, were seen after 5 hours of exposure in olfactory epithelium ($p < 0.05$), but not in the other regions. The number of IBA−1 positive-macrophages, an indicator of injury or inflammation, increased in olfactory epithelial turbinates and in the olfactory bulb after 5 hours of exposure ($p < 0.05$). Exposure for 45 hours resulted in increased oxidative stress markers, decreased levels of olfactory marker protein (expressed by mature olfactory sensory nerves), and increased levels of cleaved caspase and a related protein, PARP1, in nasal epithelium ($p < 0.05$). Caspase and PARP1 are markers of apoptosis. In olfactory bulb, oxidative stress markers were increased after 45 hours of exposure to UFP ($p < 0.05$). TNFα mRNA was increased after 20 hours and protein levels were increased after 45 hours in the nasal epithelium and olfactory bulb ($p < 0.05$). Exposure for 45 hours resulted in increased TNFα mRNA and protein in cerebral cortex and cerebellum ($p < 0.05$). CD88 mRNA was increased in olfactory bulb, as well as in cerebral cortex and cerebellum, after 20 and 45 hours of exposure ($p < 0.05$). This study demonstrated rapid responses to inhaled UFP in olfactory epithelium, and to a lesser extent, in olfactory bulb. Responses to UFP inhalation in cerebral cortex and cerebellum required longer exposures. This delay suggests a role for systemic inflammation, rather than particle translocation, in mediating the effects of UFP in these brain regions. Decreased olfactory marker protein and increased markers of apoptosis suggest an impact of UFP exposure on olfactory sensory neurons.

In addition, Allen et al. (2014b) reported changes in GFAP and IBA−1 in adult mice exposed for 4 days to UFP CAPs beginning on PND 56. Brain tissue was analyzed at 9 months. Exposure to CAPs resulted in microglial activation, measured as IBA−1 immunoreactivity, in the corpus callosum of the male mice ($p < 0.05$). A trend was observed in astrocyte activation, measured as GFAP immunoreactivity, in the cortex of the male mice. Microglial activation is an indicator of inflammation and astrocyte activation is an indicator of injury. No CAPs-related changes in either GFAP or IBA−1 were observed in the corpus callosum or cortex brain regions of female mice. Furthermore, Tyler et al. (2016) also reported changes in inflammatory markers in C67BL/6 and ApoE knockout mice exposed for 6 hours to UFP that were generated from motor vehicle exhaust. Increased mRNA levels for CCL5, CXCL1, TGF-β, and TNF-α in hippocampus of C67BL/6 mice ($p < 0.05$) and increased mRNA levels for IL−1β, IL−6, TGF-β, and TNF-α in hippocampus of ApoE knockout mice ($p < 0.05$) were observed. Minimal inflammatory effects were seen in BALF in either mouse strain although increased uptake of UFP was seen in bronchial macrophages in ApoE knockout mice (see Section 5.6.3). In contrast, exposure to UFP CAPs from Riverside, CA for 2 weeks did not induce any changes in global gene expression in the brain, or expression of Arc and Rac genes and their protein products, in Fischer 344 rats (Ljubimova et al., 2013).

Table 8-29 Study-specific details from animal toxicological studies of short-term exposure to UFP and brain inflammation and oxidative stress.

Study/Study Population	Pollutant	Exposure Details	Endpoints Examined
Allen et al. (2014b) Species: Mouse Sex: male and female Strain: C57BL/6J Age/Weight: Adult exposure at PND 56–60	CAPs collected in Rochester, NY from a "nearby highly trafficked roadway" using the Harvard University Concentrated Ambient Particle System Particle size: ≤100 nm Control: HEPA-filtered room air	Route: Whole body inhalation Dose/Concentration: 67.9 µg/m^3 Particle number: 180,000–200,000 particles/cm^3 Duration: 4 h/day, 4 days Time to analysis: 9 mo of age for brain tissue analysis	Brain tissue—Region specific levels of GFAP, IBA–1
Cheng et al. (2016) Species: Mouse Strain: C57BL/6J Sex: Male Age: 3 mo	Re-aerosolized collected ambient PM near a Los Angeles freeway Particle sizes: Ultrafine PM <180 nm Control: Re-aerosolized extracts of sham filters	Route: whole body inhalation Dose/concentration: 343 µg/m^3 Duration of exposure: 5 h/day, 3 d/week for 5, 20 and 45 h over 3 weeks	Immunohistochemistry of nasal epithelium and brain tissue • Oxidative stress markers • macrophage activation marker Protein expression in brain tissue • Cytokines • Oxidative stress markers
Ljubimova et al. (2013) Species: Rat Sex: Male Strain: Fisher 344 Age/Weight: 3–7 weeks	CAPs from Riverside, CA (summer) Particle size: <150 nm Control: Filtered air	Route: Whole body inhalation Dose/Concentration: 63 ± 8 µg/m^3 Particle number: 65,000 particles/cm^3 Duration: 5 h/day, 4 days/week for 0.5 mo	Brain tissue—Immunohistochemistry Gene expression—mRNA
Tyler et al. (2016) Species: Mouse Strain: C57BL/6 and ApoE knockout Age/Weight: 6–8 weeks	Motor vehicle exhaust (DEE and GEE) passed through a denuder to generate UFP Particle size: 147.1 nm ± 1.3 nm Control: filtered air	Route: Whole body inhalation Dose/Concentration: 371.3 ± 15.6 µg/m^3 Duration: 6 h	Hippocampal tissue: Cytokine gene expression

ApoE = apolipoprotein E; CAPs = concentrated ambient particles; DEE = diesel engine exhaust; GEE = gasoline engine exhaust; GFAP = glial fibrillary acidic protein; PND = postnatal day; IBA–1 = ionized calcium binding adaptor molecule.

8.5.4 Cognitive and Behavioral Effects

8.5.4.1 Epidemiologic Studies

Wang et al. (2014) examined the association of UFP (2-week average concentration) with depressive symptoms among older adults in the MOBILIZE study and reported findings that did support an effect of UFP on increased CESD-R score ≥ [OR=1.04 (95%CI: 0.68,1.57). Uncharacterized temporal and spatial variation in UPF concentration was an uncertainty in this study because PN concentration was measured using one monitor up to 20 km from the participant's residence.

8.5.4.2 Animal Toxicological Studies

In an animal toxicological study, Allen et al. (2013) investigated behavioral effects of short-term exposure to UFP CAPs (Table 8-30). Adult C57BL/6J mice were exposed for 4 days to UFP CAPs beginning at PND 56. Behavioral testing to evaluate responding for delayed reward was carried out. Exposure to UFP CAPs resulted in changes in mean wait time/fixed ratio completion time ($p < 0.05$), one of the behaviors related to delay of reward. Locomotor activity was evaluated and was not altered by exposure to UFP CAPs. Thus, hyperactivity was unlikely to explain the enhanced bias towards immediate rewards. When mice were exposed both postnatally (Section 8.6.5) and as adults, interactions were found for fixed ratio overall rate, fixed ratio completion time, and fixed ratio resets ($p < 0.05$).

Table 8-30 Study-specific details from animal toxicological studies of short-term UFP exposure and cognitive and behavioral effects.

Study/Study Population	Pollutant	Exposure Details	Endpoints Examined
Allen et al. (2013) Species: Mouse Sex: male and female Strain: C57BL/6J Age/Weight: Adult exposure at PND 56–60	CAPs collected in Rochester, NY from a "nearby highly trafficked roadway" using the Harvard University Concentrated Ambient Particle System Particle size: ≤100 nm Control: HEPA-filtered room air	Route: Whole body inhalation Dose/Concentration: Adult exposure mean 67.9 μg/m^3 Particle number: Mean 180,000–200,000 particles/cm^3 Duration: 4 h/day, 4 days Time to analysis: PND 71	Behavioral tests: • Preference for immediate reward • Learning/memory—novel object recognition • Locomotion

CAPs = concentrated ambient particles; HEPA = high efficiency particulate absorber; PND = postnatal day.

8.5.5 Summary and Causality Determination

The 2009 PM ISA reported limited animal toxicological evidence of a relationship between short-term exposure to UFP and nervous system effects, without supporting epidemiologic studies. Several recent experimental studies add to this evidence base. The evidence for the relationship between short-term exposure to UFP and effects on the nervous system is summarized in Table 8-31, using the framework for causality determination described in the Preamble to the ISAs (U.S. EPA, 2015).

Multi-day exposures of adult mice to UFP resulted in oxidative stress, astrocyte and microglial activation, increased cytokine levels, increased markers of apoptosis, and altered neurotransmitter levels in brain-region specific patterns (Cheng et al., 2016), (Allen et al., 2014b), (Tyler et al., 2016), (Campbell et al., 2005). Cheng et al. (2016) demonstrated the time-dependence of oxidative stress and inflammatory responses, with early changes occurring in nasal epithelium and olfactory bulb and later changes occurring in cerebellum and cerebral cortex. This finding suggests that early effects may be due to UFP translocation from nasal olfactory epithelium to olfactory bulb via olfactory sensory nerves, while later effects in more distal regions of the brain may be due to systemic inflammation. Possibly, the close proximity of the nose to the brain may enhance the ability of inflammatory mediators released by nasal epithelium to reach the brain. In addition, a controlled human exposure study links HPA stress axis activation to short-term exposure to UFP (Liu et al., 2017). Animal toxicological studies found decreases in hypothalamic norepinephrine and serum cortisol in males, but not in females, and effects on behavior related to mediating delay of reward (Allen et al., 2014b).

The strongest evidence for a relationship between short-term UFP exposure and nervous system effects is provided by animal toxicological studies that show inflammation and oxidative stress in multiple brain regions following exposure to UFP. There is a lack of evidence from epidemiologic studies because UFP is not typically measured. In addition, a study in humans found evidence for activation of the HPA stress axis in association with UFP exposure. **Overall, the collective evidence is suggestive of, but not sufficient to infer, a causal relationship between short-term UFP exposure and nervous system effects.**

Table 8-31 Summary of evidence for a suggestive of, but not sufficient to infer, a causal relationship between short-term UFP exposure and nervous system effects.

Rationale for Causality Determination[a]	Key Evidence[b]	Key References[b]	$PM_{2.5}$ Concentrations Associated with Effects[c]
Brain Inflammation and Oxidative Stress			
Evidence from multiple animal toxicological studies	Inflammation observed in several brain regions. Time-dependent changes in inflammatory and oxidative stress markers in one study	Cheng et al. (2016) Allen et al. (2014b) Tyler et al. (2016)	343 µg/m^3 67.9 µg/m^3 371.3 µg/m^3
Activation of the Hypothalamic-Pituitary-Adrenal Stress Axis			
Limited evidence from a controlled human exposure study Inconsistent evidence from an animal toxicological study	Change in level of metabolite of epinephrine/epinephrine in urine indicates HPA stress axis activation Brain region- and sex-dependent changes in norepinephrine; decreases in serum cortisol in males	Liu et al. (2017) Allen et al. (2014b)	135.8 µg/m^3 67.9 µg/m^3
Cognitive and Behavioral Effects			
Limited evidence from an animal toxicological study	Altered behavior related to mediating delay of reward which is not due to hyperactivity	Allen et al. (2013)	67.9 µg/m^3
Overall			
Lack of evidence from epidemiologic studies	Concentration data are not frequently available	Section 3.5	

[a]Based on aspects considered in judgments of causality and weight of evidence in causal framework in Table I and Table II of the Preamble.

[b]Describes the key evidence and references, supporting or contradicting, contributing most heavily to causality determination and, where applicable, to uncertainties or inconsistencies. References to earlier sections indicate where full body of evidence is characterized.

[c]Describes the $PM_{2.5}$ concentrations with which the evidence is substantiated (for experimental studies, ≤2 mg/m^3).

†Studies published since the 2009 PM ISA.

8.6 Long-term UFP Exposure and Nervous System Effects

The previous ISA reported one study involving long-term exposure to UFP. Subchronic exposure of Apo E knockout mice to UFP CAPs resulted in pro-inflammatory changes in the cortical region of the brain, including activation of cell signaling pathways and upregulation of cytokine genes (Kleinman et al.,

2008). Furthermore, magnetite UFP (10–150 nm), likely derived from combustion sources, have recently been found in frontal tissue from brains of humans (Maher et al., 2016). These findings suggest that ambient UFP may reach the brain via olfactory transport; however other routes of translocation have not been ruled out (see Chapter 4).

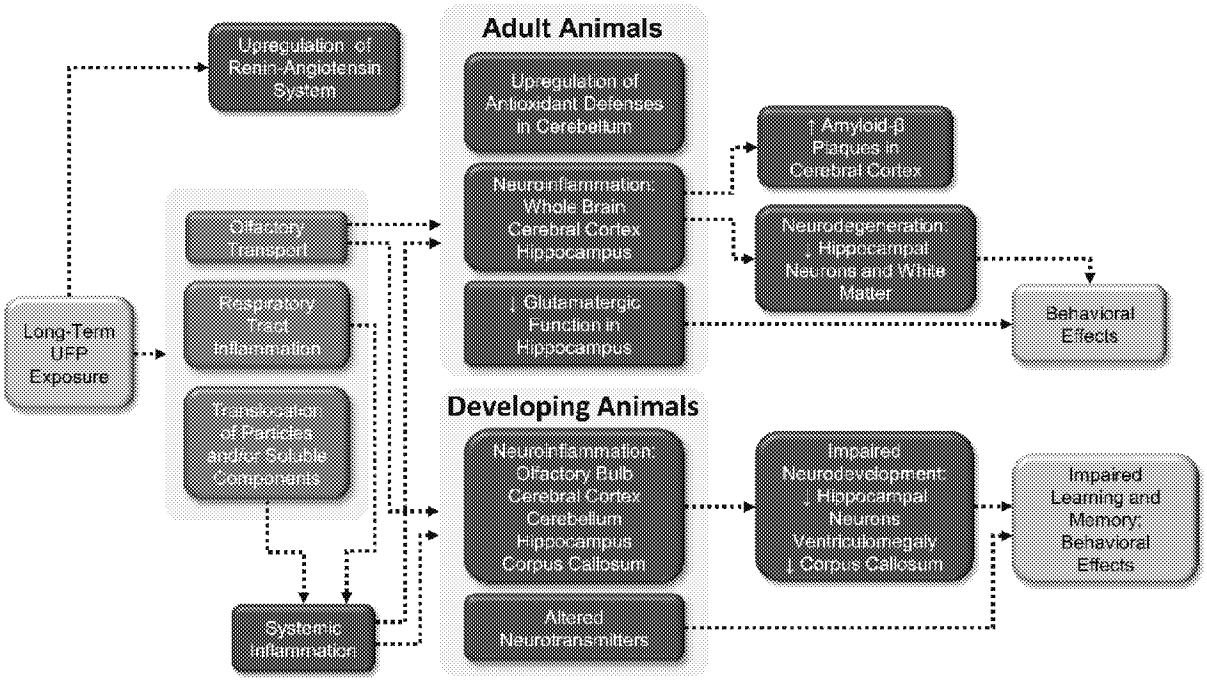
The discussion of long-term UFP exposure and nervous system effects opens with a discussion of biological plausibility (Section 8.1.1) that provides background for the subsequent sections in which groups of related endpoints are presented in the context of relevant disease pathways. These outcome groupings are activation of the SNS and HPA stress axis (Section 8.6.2), brain inflammation and oxidative stress (Section 8.6.3), morphologic changes in the brain (Section 8.6.4), cognitive and behavioral effects (Section 8.6.5) and neurodevelopmental effects (Sections 8.6.6). Finally, the collective body of evidence is integrated across and within scientific disciplines, and the rationale for the causality determination is outlined in Section 8.6.7.

8.6.1 Biological Plausibility

This section describes biological pathways that potentially underlie nervous system effects resulting from long-term exposure to UFP. Figure 8-11 graphically depicts the proposed pathways as a continuum of upstream events, connected by arrows, that may lead to downstream events observed in epidemiologic studies. This discussion of "how" long-term exposure to UFP may lead to nervous system effects contributes to an understanding of the biological plausibility of epidemiologic results evaluated later in Section 8.6.

Once UFP deposits in the respiratory tract, it may be retained, cleared, or solubilized (see Chapter 4). UFP and its soluble components may interact with cells in the respiratory tract, such as epithelial cells, inflammatory cells, and sensory nerve cells. One way in which this may occur is through reduction-oxidative (redox) reactions. As discussed in Section 2.3.3, PM may generate ROS and this capacity is termed "oxidative potential". Furthermore, cells in the respiratory tract may respond to the presence of PM by generating ROS. Further discussion of these redox reactions, which may contribute to oxidative stress, is found in Section 5.1.1 of the 2009 PM ISA (U.S. EPA, 2009). In addition, poorly soluble particles may translocate to the interstitial space beneath the respiratory epithelium and accumulate in the lymph nodes (see Chapter 4). Immune system responses due to the presence of particles in the interstitial space may contribute to health effects. Inflammatory mediators may diffuse from the respiratory tract into the systemic circulation and lead to inflammation in extrapulmonary compartments (Section 6.6.1). UFP and its soluble components may translocate into the systemic circulation and contribute to inflammatory or other processes in extrapulmonary compartments. A fraction of UFP may deposit on the olfactory epithelium. UFP and its soluble components may be transported via the olfactory nerve to the olfactory bulb of the brain. The extent to which translocation into the systemic circulation or

transport to the olfactory bulb occurs is currently uncertain. For further discussion of translocation and olfactory transport, see Chapter 4.



Note: The boxes above represent the effects for which there is experimental or epidemiologic evidence, and the dotted arrows indicate a proposed relationship between those effects. Shading around multiple boxes denotes relationships between groups of upstream and downstream effects. Progression of effects is depicted from left to right and color-coded (gray, exposure; green, initial event; blue, intermediate event; orange, apical event). Here, apical events generally reflect results of epidemiologic studies, which often observe effects at the population level. Epidemiologic evidence may also contribute to upstream boxes. When there are gaps in the evidence, there are complementary gaps in the figure and the accompanying text below.

Figure 8-11 Potential biological pathways for nervous system effects following long-term UFP exposure.

Evidence that long-term exposure to UFP may affect the nervous system generally informs one pathway (Figure 8-11). This pathway begins with pulmonary inflammation and leads to systemic inflammation and to neuroinflammation in both adult and developing animals. Neurodegeneration in adult animals and neurodevelopmental disorders in developing animals may be downstream effects of neuroinflammation and changes in neurotransmitters. Evidence for this pathway is described below.

In addition, there is evidence for two upstream events that support a possible involvement of the RAS and the SNS. Aztatzi-Aguilar et al. (2015) found upregulation of the RAS in the lung and heart in adult animals following long-term exposure to UFP (Section 5.6.3, Section 6.6.4). Allen et al. (2014b)

found increased levels of norepinephrine in the cerebral cortex and decreased levels of serum glucocorticoids in developing animals exposed to UFP postnatally. Given that the changes in RAS were observed in adult animals and the changes in norepinephrine and glucocorticoids were observed in developing animals, the relationship between these events is uncertain.

Inflammation

Deposition of UFP in the respiratory tract may lead to pulmonary inflammation (see Section 5.6.1) and to systemic inflammation (see Section 6.6.1), which in turn may lead to neuroinflammation. Neuroinflammation may be due to peripheral immune activation (Fonken et al., 2011) or to systemic circulation of UFP that results in particle uptake in the brain (Ljubimova et al., 2013). Neuroinflammation may alternatively occur following olfactory transport of poorly soluble particles or their soluble components or to a neuroendocrine stress response resulting from activation of the HPA stress axis (Kodavanti, 2016).

In adult animals, inflammatory responses were seen in whole brain, cerebral cortex, and hippocampus following long-term UFP exposure (Kleinman et al., 2008), (Morgan et al., 2011), (Cacciottolo et al., 2017), and (Tyler et al., 2016). Inflammation was accompanied by upregulation of antioxidant defense enzymes in the cerebellum (Zhang et al., 2012) and decreased markers of glutamatergic function in the hippocampus (Woodward et al., 2017). Neurodegeneration was demonstrated in the hippocampus, as indicated by decreased neurite area and decreased white matter (Woodward et al., 2017) (Cacciottolo et al., 2017). The antioxidant response, the glutamatergic response, and the neurodegeneration response were age-dependent effects that were observed in young adult rodents but not in middle-aged ones. In addition, increased amyloid-β plaques and other markers of Alzheimer's disease were seen in cerebral cortex following exposure to UFP (Cacciottolo et al., 2017). This response was dependent on the presence of several APOE alleles that are known to confer susceptibility to Alzheimer's disease. Neurodegeneration and changes in glutamatergic function occurred in conjunction with behavioral effects in adult mice exposed to UFP (Cacciottolo et al., 2017).

Neuroinflammation was also seen in developing animals exposed to UFP during the postnatal period (Allen et al., 2014a). Brain regions affected included the olfactory bulb, cerebral cortex, cerebellum, and corpus callosum. These changes occurred early after exposure and were persistent, especially in males. Morphologic changes, including ventriculomegaly, reduction in corpus callosum size, and hypomyelination of the corpus callosum were observed, especially in males (Allen et al., 2014a) (Allen et al., 2015). Postnatally-exposed rodents exhibited changes in neurotransmitters that were specific to brain region and sex (Allen et al., 2014a). Impaired learning and memory and behavioral effects were observed in developing mice exposed to UFP postnatally (Allen et al., 2014b), (Allen et al., 2013) and prenatally (Davis et al., 2013). Alterations in morphology and neurotransmitters may contribute to the observed changes in learning, memory, and behavior.

Summary of Biological Plausibility

There is one proposed pathway by which long-term UFP exposure may lead to nervous system effects. It begins with pulmonary inflammation/systemic inflammation or olfactory transport of UFP and leads to neuroinflammation. In adult animals, neuroinflammation may lead to neurodegeneration and the development of Alzheimer's disease, as well as to behavioral effects. In developing animals, neuroinflammation may lead to altered neurodevelopment and neurotransmitters. Both may contribute to impaired learning and memory and to behavioral effects. Animal toxicological and controlled human exposure studies provide the evidence for the upstream and downstream events, and there are no epidemiologic studies that evaluated the relationship between long-term UFP exposure and nervous system effects. This pathway will be used to inform a causality determination, which is discussed later in the chapter (Section 8.6.7).

8.6.2 Activation of the Sympathetic Nervous System and the Hypothalamic-Pituitary-Adrenal (HPA) Stress Axis

In an animal toxicological study, Allen et al. (2014a) investigated changes in neurotransmitters in the brains of weanling mouse pups exposed postnatally to UFP CAPs (Table 8-32). Sex-specific alterations in neurotransmitter levels were observed. In males, glutamate was increased in the hippocampus at PND 14 and 55, dopamine turnover was increased in the midbrain and cortex at PND 14 and 55, and norepinephrine was increased in the cortex at PND 55 ($p < 0.05$). In females, gamma-aminobutyric acid was reduced in the hippocampus, homovanillic acid and dopamine were increased in the midbrain, and serotonin was increased in the hippocampus at PND 14 and 55 ($p < 0.05$). In addition, norepinephrine was increased in the cortex at PND 55 ($p < 0.05$); dopamine turnover was increased in the hippocampus and reduced in the midbrain at PND 14 ($p < 0.05$).

Table 8-32 Study-specific details from an animal toxicological study of long-term exposure to UFP and activation of the sympathetic nervous system (SNS) and hypothalamic-pituitary-adrenal (HPA) stress axis.

Study/Study Population	Pollutant	Exposure Details	Endpoints Examined
Allen et al. (2014a) Species: Mouse Sex: male and female Strain: C57BL/6J Age/Weight: weanling Postnatal exposure at PND 4–7, 10–13	CAPs collected in Rochester, NY from a "nearby highly trafficked roadway" using the Harvard University Concentrated Ambient Particle System Particle size: ≤200 nm Control: HEPA-filtered room air	Route: Whole body inhalation Dose/Concentration: Prenatal exposure mean 96.4 μg/m^3 Particle number: 200,000 particles/cm^3 Duration: 4 h/day, 4 days/week Time to analysis: 24 h (PND 14) and 40 days (PND 55) after postnatal exposure or PND 270	Brain tissue—Region-specific neurotransmitter (HPLC) levels

CAPs = concentrated ambient particles; HEPA = high efficiency particulate absorber; HPLC = high performance liquid chromatograph; PND = postnatal day.

8.6.3 Brain Inflammation and Oxidative Stress

Several animal toxicological studies examined inflammatory and oxidative responses in the brains of C67BL/6J mice exposed to re-aerosolized UFP collected near a freeway in Los Angeles, CA. (Table 8-33). Morgan et al. (2011) exposed young mice (3 months) for 10 weeks and examined inflammatory responses in the cerebral cortex and the hippocampus. In the cerebral cortex, increases in mRNA of the innate immune receptor CD14 were observed in addition to increases in mRNA of the microglial marker CD68 and the astrocyte marker GFAP ($p < 0.05$). In the hippocampus, IL−1α and TNFα mRNA were increased ($p < 0.05$). Decreases in protein levels of GluA1, a glutamate receptor, were observed ($p < 0.05$), although levels of GluA2, synaptophysin, and PSD−95 were unchanged in the hippocampus. These findings indicate changes in glutamatergic functions, in addition to microglial and astrocyte activation and increased markers of inflammation.

Similarly, effects of 10-weeks exposure to UFP were studied in both young (3 months) and middle-aged (18 months) C67BL/6J mice (Woodward et al., 2017) (Zhang et al., 2012). In Cacciottolo et al. (2017), microglial activation was assessed by IBA−1 immunostaining and found to be increased in young mice, but not middle-aged mice. These changes were seen in CA1 stratum oriens and DG polymorphic layer areas of the hippocampus ($p < 0.05$) but not in the CA1 stratum radiatum, DG molecular layer, corpus callosum, and alveus. Exposure to UFP decreased by 50% the level of

glutamatergic receptor protein subunit GluA1 and increased by 10−fold TNFα mRNA in the hippocampus of young mice ($p < 0.05$). Other glutamatergic protein subunits were unaffected in young mice. Exposure to UFP had no effect on these parameters in middle-aged mice. However, age alone had an effect, with GluA1 levels decreased by 50% in middle-aged mice compared to young mice ($p < 0.05$). In Zhang et al. (2012), increases in GCLC and GCLM mRNA, as well as protein levels, were found in the cerebellum of young mice (3 months) similarly exposed ($p < 0.05$). Increases in mRNA for NAPDH quinone oxidoreductase and heme oxygenase 1 were also observed ($p < 0.05$). These Phase II regulated detoxifying enzymes are important in defense against oxidative stress. In middle-aged mice (18 months), UFP exposure resulted only in an increase in GCLM mRNA ($p < 0.05$).

Furthermore, Tyler et al. (2016) reported changes in markers related to inflammation in C57BL/6 and ApoE knockout mice exposed to UFP that was generated from motor vehicle exhaust. A 30-day exposure resulted in an increase in mRNA for CCL5 in the hippocampus of C57BL/6 mice and an increase in mRNA for CXCL1, IL−6, and TGF-β in the hippocampus of ApoE knockout mice. Minimal inflammatory effects were seen in BALF, although increased uptake of UFP was seen in bronchial macrophages (see Section 5.6.3). In contrast, exposure to UFP CAPs from Riverside, CA for 2 weeks did not induce any changes in global gene expression in the brain, or expression of Arc and Rac genes and their protein products, in Fischer 344 rats (Ljubimova et al., 2013).

Table 8-33 Study-specific details from animal toxicological studies of long-term exposure to UFP and brain inflammation and oxidative stress.

Study/Study Population	Pollutant	Exposure Details	Endpoints Examined
Ljubimova et al. (2013) Species: Rat Sex: Male Strain: Fisher 344 Age/Weight: 3−7 weeks	CAPs from Riverside, CA (summer) Particle size: <150 nm Control: Filtered air	Route: Whole body inhalation Dose/Concentration: 63 µg/m³ Particle number: 65,000 particles/cm³ Duration: 5 h/day, 4 days/week for 1, 3, and 10 mo	Brain tissue—Immunohistochemistry Gene expression—mRNA
Morgan et al. (2011) Species: Mouse Strain: C57Bl/6J Sex: Male Age: 3 mo	Re-aerosolized collected ambient PM near a freeway Particle sizes: Ultrafine PM <180 nm Control: Re-aerosolized extracts of sham filters	Route: whole body inhalation Dose/concentration: 468 ± 25 µg/m³ 254,000 particles/cm³ Duration of exposure: 5 h/day, 3 days/week for 10 weeks	Expression of hippocampal proteins • GLuA1, GluA2, synaptophysin and PSD95 Glial activation—mRNA of microglial markers CD14 and CD68, astrocyte GFAP cytokines

Table 8-33 (Continued): Study-specific details from animal toxicological studies of long-term exposure to UFP and brain inflammation and oxidative stress.

Study/Study Population	Pollutant	Exposure Details	Endpoints Examined
Cacciottolo et al. (2017) Species: Mouse Strain: C57Bl/6J Sex: Female Age: 3 and 18 mo	Re-aerosolized collected ambient PM near a freeway, mixed with HEPA-filtered air Particle sizes: Ultrafine PM < 180 nm Control: HEPA-filtered air	Route: whole body inhalation Dose/concentration: 468 ± 25 μg/m^3 254,000 particles/cm^3 Duration of exposure: 5 h/day, 3 days/week for 10 weeks	Expression of hippocampal proteins • GLuA1, GluA2, and other synaptic proteins Microglial activation—IBA−1 immunostaining
Tyler et al. (2016) Species: Mouse Strain: C57BL/6 and ApoE knockout Age/Weight: 6−8 weeks	Motor vehicle exhaust (DEE and GEE) passed through a denuder to generate UFP Particle size: 147.1 nm ± 1.3 nm Control: filtered air	Route: Whole body inhalation Dose/Concentration: 371.3 ± 15.6 μg/m^3 Duration: 6 h/day for 30 days	Hippocampal tissue: Cytokine gene expression
Zhang et al. (2012) Species: Mouse Strain: C57BL/6J Sex: Male Age: 3 mo, 18 mo	Re-aerosolized collected ambient PM near a freeway Particle sizes: Ultrafine PM <200 nm Control: Re-aerosolized extracts of sham filters	Route: whole body inhalation Dose/concentration: 300−400 μg/m^3 Duration of exposure: 5 h/day, 3 day/week for 10 weeks	Oxidative stress markers—Cerebellar GCLC, GCLM, heme oxygenase−1, and NADPH quinone oxidoreductase mRNA and protein

ApoE = apolipoprotein E; CAPs = concentrated ambient particles; CD = cluster of differentiation; DEE = diesel engine exhaust; GEE = gasoline engine exhaust; GCLC = glutamate-cysteine ligase catalytic subunit; GCLM = glutamate-cysteine ligase modifier subunit; GFAP = glial fibrillary acidic protein; Glu = glutamate; HEPA = high efficiency particulate absorber; IBA−1 = ionized calcium-binding adapter molecule 1; NADPH = nicotinamide adenine dinucleotide phosphate reduced form; PSD = postsynaptic density protein.

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8.6.4 Morphologic Changes

2 Animal toxicological studies investigated morphologic changes in the brain following long-term
3 UFP exposure (Table 8-34). Effects of a 10-week exposure to UFP collected from a Los Angeles freeway
4 on brain morphology were evaluated in both young (3 months) and middle-aged (18 months) C67BL/6J
5 mice (Cacciottolo et al., 2017). Exposure to UFP decreased neurite area in specific hippocampal regions
6 of young mice (i.e., the stratum oriens and stratum radiatum CA1 regions but not the DG or CA3 regions,
7 $p < 0.05$). No changes in neurite area were seen in the forceps major of the corpus callosum or

hippocampal alveus in young mice or in any of the examined areas in middle-aged mice as a result of UFP exposure. Changes in white matter were assessed by staining for myelin basic protein. Middle-aged mice had decreased myelin basic protein in specific hippocampal regions, (i.e., CA1 stratum oriens and DG polymorphic layer compared with young mice, $p < 0.05$). Exposure to UFP resulted in changes in myelin basic protein in the hippocampal stratum oriens of young mice ($p < 0.05$). No UFP exposure-related changes were seen in middle-aged mice. However, age alone had an effect, with myelin basic protein decreased by 50% in the CA1 striatum oriens and 45% in the DG polymorph layer of the hippocampus of middle-aged mice compared with young mice ($p < 0.05$).

Using the same exposure system, Cacciottolo et al. (2017) examined the effect of UFP exposure and the presence of APOE alleles on the development of pathology related to Alzheimer's disease in mice. In wild type mice, 10-weeks inhalation of UFP resulted in decreased neurite density in the hippocampus at 7 months of age. This involved selective loss of hippocampal CA1 neurons ($p < 0.005$) but not DG neurons. In addition, the density of GluR1 receptor subunits, but not other synaptic proteins involved in hippocampal-based memory, was decreased in the hippocampus of wild type mice ($p < 0.005$). In mice carrying transgenes for human APOE ϵ3 or ϵ4 alleles in combination with five familial AD mutations (EFAD mice), similar changes were observed at 7 months of age following 15-weeks inhalation of UFP ($p < 0.01$). These changes were not dependent on the number of alleles (E3FAD vs E4FAD). However, exposure to UFP resulted in increases in amyloid deposits in the cerebral cortex of E4FAD mice but not E3FAD mice ($p < 0.05$). Similarly, amyloid-β oligomers in soluble extracts of cerebral cortex were increased in E4FAD mice but not E3FAD mice ($p < 0.05$). APOE alleles are known to confer susceptibility to Alzheimer's disease which is characterized by the accumulation of amyloidβ and cognitive effects. APOE ϵ4 confers greater susceptibility to women than men. While EFAD mice are known to accumulate amyloid aggregates at an early age, wild type C67Bl/6J do not develop amyloid aggregates at any age.

Table 8-34 Study-specific details from animal toxicological studies of long-term exposure to UFP and morphologic changes.

Study/Study Population	Pollutant	Exposure Details	Endpoints Examined
Cacciottolo et al. (2017) Strain: C57BL/6J and EFAD mice carrying transgenes for human APOE ϵ3 or ϵ4 alleles in combination with five familial AD mutations Sex: Female Age: 8 weeks	Re-aerosolized collected ambient PM near a freeway Particle sizes: Ultrafine PM <200 nm Control: Re-aerosolized extracts of sham filters	Route: whole body inhalation Dose/concentration: 468 ± 25 μg/m^3 254,000 particles/cm^3 Duration of exposure: 5 h/day, 3 days/week for 15 weeks (transgenic mice) or 10 weeks (wild type mice) Time to analysis: 7 mo of age	Brain tissue—Immunohistochemistry Histochemistry Protein levels Immunoassay
Woodward et al. (2017) Species: Mouse Strain: C57BL/6J Sex: Female Age: 3 and 18 mo	Re-aerosolized collected ambient PM near a freeway, mixed with HEPA-filtered air Particle sizes: Ultrafine PM <180 nm Control: HEPA-filtered air	Route: whole body inhalation Dose/concentration: 342 ± 49 μg/m^3 140,000 particles/cm^3 Duration of exposure: 5 h/day, 3 days/week for 10 weeks	Histochemistry: Hippocampus neurite area and Myelin Basic Protein

AD = Alzheimer's disease; APOE = apolipoprotein E; EFAD = early onset familial Alzheimer disease; HEPA = high efficiency particulate absorber.

8.6.5 Cognitive and Behavioral Effects

An animal toxicological study investigated cognitive and behavioral effects following long-term UFP exposure (Table 8-35). Effects of a 10-week exposure to UFP collected from a Los Angeles freeway were studied in both young (3 months) and middle-aged (18 months) C67BL/6J mice (Cacciottolo et al., 2017). There were no age- or UFP exposure-related changes in short- or long-term memory, as assessed by the novel object recognition test, or in working memory, as assessed by the spontaneous alternation of behavior test. However, UFP exposure decreased exploratory behavior by 30% ($p < 0.01$) in middle-aged mice and activity in both age groups ($p < 0.05$). Middle aged mice also responded to UFP exposure with weight loss ($p < 0.05$) that was reversible upon cessation of exposure and that correlated with changes in locomotor activity ($p < 0.05$).

Table 8-35 Study-specific details from an animal toxicological study of long-term exposure to UFP and cognitive and behavioral effects.

Study/Study Population	Pollutant	Exposure Details	Endpoints Examined
Cacciottolo et al. (2017) Species: Mouse Strain: C57BL/6J Sex: Female Age: 3 and 18 mo	Re-aerosolized collected ambient PM near a freeway, mixed with HEPA-filtered air Particle sizes: Ultrafine PM <180 nm Control: HEPA-filtered air	Route: whole body inhalation Dose/concentration: 468 ± 25 μg/m^3 254,000 particles/cm^3 Duration of exposure: 5 h/day, 3 days/week for 10 weeks	Tests of cognition and activity

HEPA = high efficiency particulate absorber.

8.6.6 Neurodevelopmental Effects

8.6.6.1 Epidemiologic Studies

Sunyer et al. (2015) enrolled students (n = 2,715, 7−10 years old) from 39 schools in Barcelona, Spain in order to study the relationship between cognitive development and traffic related pollutants including UFP (Table 8-36). Schools were selected from high and low pollution areas and matched by school socioeconomic index. The study was longitudinal in design with repeated cognitive testing during an approximately one-year period. The outcomes, validated tests of working memory and attention, were selected because they measure cognitive functions that are typically under development during the lifestages of the children participating (i.e., 7−10 years old). Authors reported a 12 month decrease in both working [−4.9 (95% CI: −10, 0.22) per IQR increase in UFP] and superior working memory [−5 (95% CI: −9.1, −0.96) per IQR Increase in UFP]. A 12 month increase in inattentiveness was also reported [3.9 (0.31, 7.6) per IQR increase in UFP].

Table 8-36 Characteristics of the studies examining the association between long-term exposure to UFP and neurodevelopmental effects.

Study Location/Years	Study Population	Exposure Assessment	Concentration	Outcome	Copollutant Examination
†Sunyer et al. (2015) Barcelona, Spain Jan 2012–March 2013 Longitudinal Cohort	School children 7–10 yr N = 2,715 39 schools	Direct measurement of UFP (10–700 nm) at schools. 2 times during 1-week periods separated by 6 mo to reflect warm and cold seasons	UFP Outdoor: 22,157 particles per cubic cm	Working memory and attention	Copollutant correlations (r): EC outdoors $r = 0.62$ Copollutant model: NR

Mo=month(s); N, n = number of subjects; nm=nanometers; NR=not reported; yr=year(s).

†Studies published since the 2009 PM ISA.

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8.6.6.2 Animal Toxicological Studies

Several animal toxicological studies examined the effects of long-term UFP exposure on neurodevelopment (Table 8-37). Davis et al. (2013) measured markers of glutamate receptors, neuronal growth cones, synaptic proteins, kinases, and glial proteins in the hippocampus of young C57BL/6J mice exposed prenatally to UFP collected from a Los Angeles freeway. Dams were exposed to UFP prior to conception, mated with unexposed males, and then exposed to UFP during gestation. Thus, exposure occurred throughout oocyte maturation and gestation. Prenatal exposure to UFP resulted in a decrease in protein levels of JNK1, a protein kinase, in the hippocampus of neonatal offspring ($p \leq 0.05$). Many markers of inflammation and other processes were unchanged. Davis et al. (2013) also investigated internalizing disorders using specific behavioral testing in the offspring. Male offspring exhibited behavioral sequelae, with decreased latency to immobility and increased duration of immobility in the tail-suspension test ($p < 0.05$), a test of propensity for mental health impairment or depression and low resilience to stress; females were refractory to change with these endpoints. Female and male offspring did not display changes in tests of anxiety. Prenatal UFP exposure was associated with changes in internalizing behavior of depression but not anxiety in male offspring; internalizing behavior of female offspring was not affected by prenatal UFP exposure.

Allen et al. (2015); Allen et al. (2014a) investigated the effects of exposure to UFP CAPs in weanling mouse pups during PND 4−7 and PND 10−13. This post-gestational time period, which is considered equivalent to the third trimester in humans, is marked by rapid neuro- and gliogenesis. Mice were sacrificed at PNDs 14, 55, and 270. UFP CAPs exposure altered GFAP immunostaining, an indicator of astrocyte activation, in a sex-specific manner. GFAP immunostaining was reduced in the hippocampus of male mice at PND 14 and in the corpus callosum of male mice at PND 14 and PND 55 ($p < 0.05$). However, GFAP was increased at PND 14 in the amygdala ($p \leq 0.05$). In females, GFAP immunostaining increased in hippocampus, corpus callosum, and anterior commissure on PND 14 ($p < 0.05$), but not on PND 55. UFP CAPs exposure also altered IBA−1 immunostaining, an indicator of glial activation, in a sex-specific manner. In males, IBA−1 immunostaining was increased in the anterior commissure at PND 14 and PND 55, in the hippocampus at PND 55, and in the corpus callosum at PND 270 ($p < 0.05$). No changes were seen in females. Findings of early (astrocyte and microglial) and persistent (microglial) activation, especially in males, suggest that astrocyte and microglial activation may be important mediators of responses to UFP CAPs exposure.

Allen et al. (2014a) and Allen et al. (2015) also examined morphologic changes in the brains of these weanling mouse pups exposed postnatally to UFP CAPs. Ventriculomegaly was observed in PND 14 male ($p \leq 0.05$), but not female mice. This effect in male mice persisted in young adulthood (PND 55) and at PND 270 ($p \leq 0.05$). Ventriculomegaly is related to poor neurodevelopmental outcomes in children, which tend to be higher in males. In addition, exposure to UFP CAPs resulted in a reduction in

the size of the corpus callosum in both sexes at PND 14 ($p \leq 0.05$) and a male-specific decrease in myelination in the corpus callosum at PND 14 ($p \leq 0.05$). Striatal and frontal cortex myelination was unaffected by exposure to UFP CAPs in either sex. Findings of ventriculomegaly, reductions in corpus callosum size, and hypomyelination, especially in males, are consistent with morphologic changes associated with neurodevelopmental disorders such as ASD in humans.

Allen et al. (2013) and Allen et al. (2014b) investigated behavioral effects in male and female mice exposed to UFP CAPs, as described above. Behavioral testing was carried out on PND 71 and animals were sacrificed one month later. Some mice were exposed a second time to UFP CAPs beginning at PND 56 for 4 days. In the first study, Allen et al. (2013) found that postnatal exposure to UFP CAPs resulted in enhanced preference for immediate reward. This was evidenced by changes in fixed ratio overall rate, run rate, inter-response time, fixed ratio resets, and responses per reinforcer ($p < 0.05$). Additionally, interactions were found for fixed ratio overall rate, fixed ratio completion time, and fixed ratio resets ($p < 0.05$) in mice that were exposed both postnatally and as adults. Locomotor activity was evaluated and found to not be altered by exposure to UFP CAPs, indicating that hyperactivity was unlikely to explain the behavioral alterations. In the second study, Allen et al. (2014b) measured initial fixed interval schedule controlled behavior, which is related to preference for immediate reward, and a measure of impulsivity. Novel object recognition, which is an indicator of learning and short-term memory, and locomotor activity were also determined. Postnatal exposure to UFP CAPs resulted in greater impulsivity-linked behavior. In males, postnatal exposure resulted in decreases in overall rate and run rate ($p < 0.05$) while in females, adult exposure resulted in increases in overall rate and run rate ($p < 0.05$). Indices of novel object recognition were decreased by postnatal UFP CAPs exposure in male (change in time with novel object) and female (change in time/approaches to novel object) mice ($p < 0.05$). Interactions resulting from exposure during both the postnatal and adult lifestage were noted for both sets of behavioral tests. Spontaneous locomotor behavior was impaired in both males and females as a result of exposure to UFP CAPs during both lifestages ($p < 0.05$). Furthermore, levels of serum corticosterone and some brain region-specific neurotransmitters were correlated with measures of impulsivity-linked behavior in male mice exposed during the postnatal period and in female mice exposed as adults ($p < 0.05$).

Altogether, these results indicate that prenatal and postnatal exposure to UFP CAPs led to neurotoxic changes which persisted over time. These effects included neuroinflammation, morphologic changes, and behavioral effects.

Table 8-37 Study-specific details from animal toxicological studies of long-term exposure to UFP and neurodevelopmental effects.

Study/Study Population	Pollutant	Exposure Details	Endpoints Examined
Allen et al. (2013) Species: Mouse Sex: male and female Strain: C57BL/6J Age/Weight: weanling Postnatal exposure at PND 4–7, 10–13 Adult exposure at PND 56–60	CAPs collected in Rochester, NY from a "nearby highly trafficked roadway" using the Harvard University Concentrated Ambient Particle System Particle size: ≤200 nm Control: HEPA-filtered room air	Route: Whole body inhalation Dose/Concentration: Prenatal exposure mean 96.4 µg/m^3 Adult exposure mean 67.9 µg/m^3 Particle number: Mean 180,000–200,000 particles/cm^3 Duration: 4 h/day, 4 days/week Time to analysis: 24 h after final exposure-PND 14	Behavioral tests • Preference for immediate reward • Learning/memory—novel object recognition • Locomotion
Allen et al. (2014b) Species: Mouse Sex: male and female Strain: C57BL/6J Age/Weight: weanling Postnatal exposure at PND 4–7, 10–13 Adult exposure at PND 56–60	CAPs collected in Rochester, NY from a "nearby highly trafficked roadway" using the Harvard University Concentrated Ambient Particle System Particle size: ≤200 nm Control: HEPA-filtered room air	Route: Whole body inhalation Dose/Concentration: Prenatal exposure mean 96.4 µg/m^3 Adult exposure mean 67.9 µg/m^3 Particle number: 180,000–200,000 particles/cm^3 Duration: 4 h/day, 4 days/week Time to analysis: PND 71 for behavioral testing 9 mo of age for brain tissue analysis PND 60 and 6 mo of age for blood collection	Behavioral tests Impulsivity—fixed interval schedule-controlled performance Learning/memory—novel object recognition Locomotion Brain tissue—Region specific levels of monoamines, amino acids, GFAP, IBA–1 Blood—corticosterone
Allen et al. (2014a) Species: Mouse Sex: male and female Strain: C57BL/6J Age/Weight: weanling Postnatal exposure at PND 4–7, 10–13	CAPs collected in Rochester, NY from a "nearby highly trafficked roadway" using the Harvard University Concentrated Ambient Particle System Particle size: ≤200 nm Control: HEPA-filtered room air	Route: Whole body inhalation Dose/Concentration: Prenatal exposure mean 96.4 µg/m^3 Particle number: 200,000 particles/cm^3 Duration: 4 h/day, 4 days/week Time to analysis: 24 h (PND14) and 40 days (PND 55) after postnatal exposure or PND 270	Immunostaining—GFAP and IBA–1 Image analysis Brain tissue—Region-specific cytokine (immunoassay) levels
Allen et al. (2015) Species: Mouse Sex: male and female Strain: C57BL/6J Age/Weight: weanling Postnatal exposure at PND 4–7, 10–13	CAPs collected in Rochester, NY from a "nearby highly trafficked roadway" using the Harvard University Concentrated Ambient Particle System Particle size: ≤200 nm Control: HEPA-filtered room air	Route: Whole body inhalation Dose/Concentration: Mean 96 µg/m^3 Particle number: 200,000 particles/cm^3 Duration: 4 h/day, 4 days/week Time to Analysis: PNDs 14, 55, 270	Immunostaining—brain tissue Image analysis—brain tissue

Table 8-37 (Continued): Study-specific details from animal toxicological studies of long-term exposure to UFP and neurodevelopmental effects.

Study/Study Population	Pollutant	Exposure Details	Endpoints Examined
Davis et al. (2013) Species: Mouse Strain: C57BL/6J Sex: Female Age: 3 mo	Re-aerosolized collected ambient PM near a freeway Particle Sizes: Ultrafine PM <180 nm, Control: Re-aerosolized extracts of sham filters	Route: whole body inhalation Dose/Concentration: 350 μg/m^3 Duration of exposure: 5 h/day, 3 day/week for 7 weeks before conception and through gestation up to 2 days before birth Time to analysis: PND 3 for brain tissue 8 mo for behavioral testing	Expression of hippocampal proteins • markers of glutamate receptors, neuronal growth cones, synaptic proteins, kinases and glial proteins Behavioral testing • tail suspension test Preliminary physical assessment

CAPs = concentrated ambient particles; GFAP = glial fibrillary acidic protein; IBA−1 = ionized calcium binding adaptor molecule 1; HEPA = high efficiency particulate absorber; PND = postnatal day.

8.6.7 Summary and Causality Determination

The 2009 PM ISA reported limited animal toxicological evidence of a relationship between long-term exposure to UFP and nervous system effects, without supporting epidemiologic studies. Recent animal toxicological studies substantially add to this evidence base by demonstrating neuroinflammation, Alzheimer's disease-related pathology, neurodegeneration, and altered neurodevelopment. Recent epidemiologic studies are very limited in number. The evidence for the relationship between long-term exposure to UFP and effects on the nervous system is summarized in Table 8-38, using the framework for causality determination described in the Preamble to the ISAs (U.S. EPA, 2015).

Studies of long-term exposure of adult mice to UFP from traffic-dominated sources provide evidence of inflammation and oxidative stress in the whole brain, hippocampus, and cerebral cortex (Cacciottolo et al., 2017; Tyler et al., 2016; Zhang et al., 2012; Morgan et al., 2011; Kleinman et al., 2008). Astrocyte activation and altered glutamatergic functions were also seen in these studies. Neurodegeneration, as indicated by decreased neurite density and white matter, occurred in specific regions of the hippocampus in UFP exposed mice (Cacciottolo et al., 2017). Many responses, including neurodegeneration, were greater in young compared with middle-aged mice. However, one of the measured behavioral effects was altered to a greater degree by UFP exposure in middle-aged mice compared with young mice (Cacciottolo et al., 2017). Pathologic changes characteristic of Alzheimer's disease (i.e., amyloid deposits and amyloid-β oligomers in the cortex) were seen in a mouse model of Alzheimer's disease, but not in wild type mice following exposure to UFP (Cacciottolo et al., 2017).

Prenatal exposure to UFP resulted in altered behavioral indices in adult male, but not female, mice (Davis et al., 2013). Postnatal exposure to UFP CAPs led to developmental neurotoxicity in a group of studies from the same laboratory (Allen et al., 2015; Allen et al., 2014b; Allen et al., 2014a; Allen et al., 2013). Activation of microglia and astrocytes, indicative of inflammation and injury, respectively, was observed along with alterations in brain morphology and neurotransmitters, and changes in serum corticosterone and behavior. Some effects were sex-specific, notably the persistent ventriculomegaly found in male mice (Allen et al., 2015; Allen et al., 2014a). Long-term exposure to UFP was associated with effects on cognitive development in children (Sunyer et al., 2015). However, uncertainties remain as a result of inadequate assessment of potential copollutant confounding, the spatial variation in UFP concentrations, and exposure measurement error.

The strongest evidence is provided by animal toxicological studies showing inflammation, oxidative stress, and neurodegeneration in adult mice and Alzheimer's disease pathology in a susceptible animal model. In addition, pre- and early postnatal exposure to UFP results in behavioral effects, inflammation, and persistent morphologic changes. Epidemiologic studies of UFP were lacking. **Overall, the collective evidence is sufficient to conclude that a causal relationship is likely to exist between long-term UFP exposure and nervous system effects.**

Table 8-38 Summary of evidence for a likely to be causal relationship between long-term UFP exposure and nervous system effects.

Rationale for Causality Determination[a]	Key Evidence[b]	Key References[b]	$PM_{2.5}$ Concentrations Associated with Effects[c]
Brain Inflammation and Oxidative Stress			
Consistent evidence from multiple toxicological studies	Evidence of inflammation in whole brain, cerebral cortex, and hippocampus; evidence of oxidative stress in cerebellum	(Kleinman et al., 2008) †(Morgan et al., 2011) †(Cacciottolo et al., 2017) †(Tyler et al., 2016) †(Zhang et al., 2012)	114.2 µg/m^3 468 µg/m^3 342 49 µg/m^3 371.3 µg/m^3 200–400 µg/m^3
Activation of the Sympathetic Nervous System			
Inconclusive evidence	Changes in norepinephrine in cortex but levels in hypothalamus were not determined	†(Allen et al., 2014a)	96.4 µg/m^3

Table 3-38 (Continued): Summary of evidence for a likely to be causal relationship between long-term exposure to ultrafine particulate and nervous system effects.

Rationale for Causality Determination[a]	Key Evidence[b]	Key References[b]	$PM_{2.5}$ Concentrations Associated with Effects[c]
Morphologic Changes			
Evidence from animal toxicological studies	Neurodegenerative changes in hippocampus	†(Cacciottolo et al., 2017)	342 µg/m^3
		†(Cacciottolo et al., 2017)	468 µg/m^3
	Alzheimer's disease pathology in cerebral cortex; dependent on APOE alleles	†(Cacciottolo et al., 2017)	468 µg/m^3
Cognitive and Behavioral Effects			
Limited animal toxicological evidence	Behavioral effects in adult mice	†(Cacciottolo et al., 2017)	342 ± 49 µg/m^3
Neurodevelopmental Effects			
Extensive evidence from animal toxicological studies from two different laboratories	Behavioral effects resulting from prenatal and postnatal exposure	†(Davis et al., 2013)	350 µg/m^3
		†(Allen et al., 2014b)	96.4 µg/m^3
	Altered neurotransmitters	†(Allen et al., 2013)	96.4 µg/m^3
		†(Allen et al., 2014a)	96.4 µg/m^3
	Neuroinflammation and morphologic changes including persistent morphology resulting from postnatal exposure	†(Allen et al., 2014b)	96.4 µg/m^3
		†(Allen et al., 2014a)	96.4 µg/m^3
		†(Allen et al., 2015)	96.4 µg/m^3
Overall			
Limited epidemiologic evidence	Associations with increased inattention and decreased scores on tests of memory	†(Sunyer et al., 2015)	22,157 particles/cubic cm
Uncertainty regarding copollutant confounding	No copollutant model results were reported.		
Uncertainty due to exposure measurement error	UFP concentration data for use in epidemiologic studies not frequently available; where available spatial variation of UFP may remain uncharacterized	Section 3.5	

[a]Based on aspects considered in judgments of causality and weight of evidence in causal framework in Table I and Table II of the Preamble.

[b]Describes the key evidence and references, supporting or contradicting, contributing most heavily to causality determination and, where applicable, to uncertainties or inconsistencies. References to earlier sections indicate where full body of evidence is characterized.

[c]Describes the $PM_{2.5}$ concentrations with which the evidence is substantiated (for experimental studies, ≤2 mg/m^3).

†Studies published since the 2009 PM ISA.

8.7 References

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CHAPTER 9 REPRODUCTIVE AND DEVELOPMENTAL EFFECTS

Summary of Causality Determinations for Particulate Matter (PM) Exposure and Male and Female Reproduction and Fertility, and Pregnancy and Birth Outcomes

This chapter characterizes the scientific evidence that supports causality determinations for short- and long-term PM exposure and reproductive and developmental outcomes. The types of studies evaluated within this chapter are consistent with the overall scope of the ISA as detailed in the Preface (Section 11P.3.1). In assessing the overall evidence, strengths and limitations of individual studies were evaluated based on scientific considerations detailed in the Appendix. The evidence presented throughout this chapter support the following causal conclusions. More details on the causal framework used to reach these conclusions are included in the Preamble to the ISA (U.S. EPA, 2015).

Size Fraction	Causality Determination
Male and Female Reproduction and Fertility	
$PM_{2.5}$	Suggestive of, but not sufficient to infer
$PM_{10-2.5}$	Inadequate to infer
UFP	Inadequate to infer
Pregnancy and Birth Outcomes	
$PM_{2.5}$	Suggestive of, but not sufficient to infer
$PM_{10-2.5}$	Inadequate to infer
UFP	Inadequate to infer

This chapter evaluates the scientific evidence related to the potential effects of PM ($PM_{2.5}$, $PM_{10-2.5}$, and ultrafine particles [UFP]) on reproductive and developmental outcomes in three sections including (1) Male and Female Reproduction and Fertility; (2) Pregnancy and Birth Outcomes; and (3) Developmental Effects. The body of literature characterizing reproductive and developmental effects associated with exposure to PM is large and has grown considerably since the 2009 PM ISA (U.S. EPA, 2009). Well-designed studies with consideration of potential confounding and other sources of bias are emphasized in this section (see APPENDIX 1 for study evaluation guidelines). In order to evaluate and characterize the evidence for the effects of PM on reproductive and developmental effects in a consistent, cohesive and integrated manner, results from both short-term and long-term exposure periods are included in a single section and are identified accordingly in the text and tables throughout this section. Because

the length of gestation in rodents is 18–24 days, on average, animal toxicological studies investigating the effects of PM generally are short-term exposure periods. For comparison, an epidemiologic study that uses the entire pregnancy as the exposure period is considered to have a long-term exposure period (about 40 weeks, on average). A major issue in studying environmental exposures and reproductive and developmental effects (including infant mortality) is selecting the relevant exposure period, since the biological plausibility leading to these outcomes and the critical periods of exposure are not completely understood. Thus, multiple exposure periods are evaluated in many epidemiologic studies, including long-term (months to years) exposure periods, such as entire pregnancy, individual trimesters or months of pregnancy, and short-term (days to weeks) exposure periods such as the days and weeks immediately preceding birth. Thus, the biological plausibility for the effects of PM on reproductive and developmental outcomes will combine short-term and long-term exposures in each particle size class ($PM_{2.5}$, UFP, and coarse PM). Further, infants and fetal development processes may be particularly sensitive to PM exposure, and although the physical mechanisms are not always fully understood the impacts from PM exposure at these critical windows of development may have permanent, lifelong effects.

Separate causality determinations are made for the two sections Male and Female Fertility and Reproduction; Pregnancy and Birth Outcomes. For developmental effects, summaries are included in this section of the ISA and full descriptions as well as causality determinations are found in the specific health endpoint (respiratory, cardiovascular, metabolic and neurological disease) section.

9.1 $PM_{2.5}$ Exposure and Reproductive and Developmental Effects

The body of literature characterizing male and female reproduction and fertility with $PM_{2.5}$ exposure is large and has grown considerably since the 2009 PM ISA (U.S. EPA, 2009). The evidence from the 2009 PM ISA determined that there was a suggestive causal relationship between long-term $PM_{2.5}$ exposure and reproductive and developmental outcomes. Effects of $PM_{2.5}$ exposure on sperm have been studied in both the animal toxicology and the epidemiologic literature. The strongest effects in the epidemiologic literature come from studies on sperm motility with $PM_{2.5}$ associated with impaired motility. The toxicological literature also has $PM_{2.5}$-dependent effects on sperm including impaired spermatogenesis and spermiation. Other studies from epidemiologic literature on sperm morphology have inconsistent results. Studies of female reproduction in association with $PM_{2.5}$ exposure cover estrus, ovulation, reproduction, and fertility. In rodents, ovulation and estrus are affected by $PM_{2.5}$ exposure. In the epidemiologic literature, results on human fertility and fecundity in association with $PM_{2.5}$ exposure is limited, but evidence from IVF shows a modest association of $PM_{2.5}$ concentrations with decreased odds of becoming pregnant. The toxicological evidence provides biological plausibility to these outcomes and shows multiple sensitive windows for PM exposure's effects. In the pregnancy and birth outcomes section of this document, studies on fetal growth, birth weight, preterm birth and preterm rupture of membranes show positive associations with $PM_{2.5}$ exposure in some animal toxicology and epidemiologic studies.

The toxicological evidence gives biological plausibility to these outcomes and shows multiple sensitive windows for PM exposure's effect on pre-term birth and low birth weight. Multiple epidemiologic and toxicological studies of birth defects show that PM is associated with cardiovascular birth defects, albeit of different types. The studies of fetal growth, birth weight, and infant mortality, increased in number in this ISA but generally continue to lack controls for confounding by other air pollutants, and show sensitivity to PM exposure across multiple trimesters of the pregnancy. Studies on sperm had mixed effects with epidemiologic studies of sperm focused on motility and toxicological studies focused on spermatogenesis. Studies of fertility in females showed effects on estrus in animal toxicology studies. Pregnancy outcomes showed mixed effects with $PM_{2.5}$ exposure and gestational diabetes, but when analyzed by trimester, the 2nd trimester showed the strongest effects, especially with gestational diabetes. In animal toxicological studies, the structure and vascularization of the placenta and umbilical cord were affected by $PM_{2.5}$ exposure. Developmental outcomes included cardiovascular, respiratory, and neurological outcomes like autism and are covered in more detail in those respective sections. More detailed information on male and female reproduction and fertility, pregnancy and birth outcomes, and developmental effects follows below.

9.1.1 Male and Female Reproduction and Fertility

9.1.1.1 Biological Plausibility

This section describes biological pathways that potentially underlie reproductive and developmental health effects specific to male and female reproduction and fertility resulting from exposure to $PM_{2.5}$. Figure 9-1 graphically depicts the proposed pathways as a continuum of upstream events, connected by arrows, that may lead to downstream events observed in epidemiologic studies. This discussion of "how" exposure to $PM_{2.5}$ may lead to effects on Reproduction and Fertility contributes to an understanding of the biological plausibility of epidemiologic results evaluated later in Section 9.1.

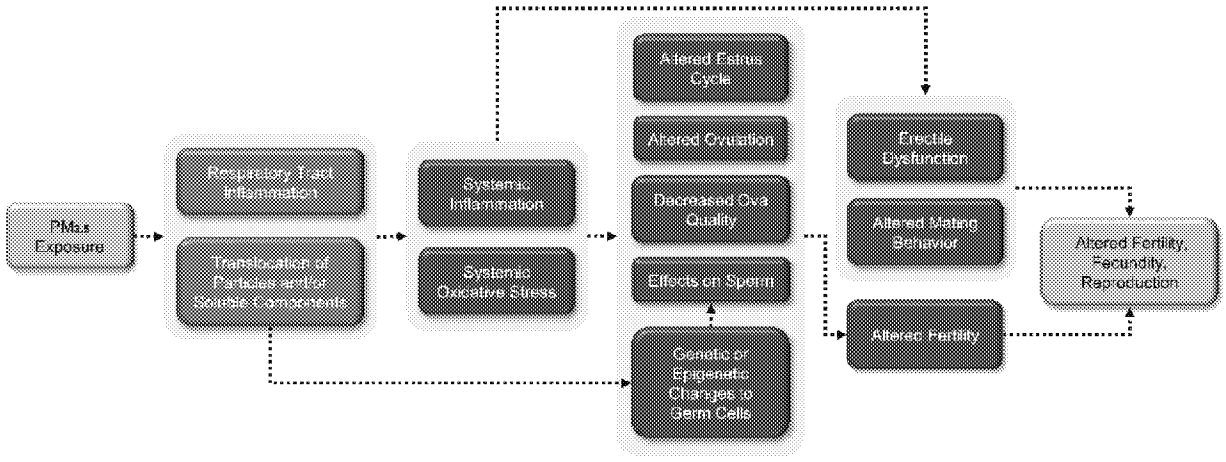


Figure 9-1 Potential biological pathways for male and female reproduction and fertility effects following $PM_{2.5}$ exposure

[a] Note: The boxes above represent the effects for which there is experimental or epidemiologic evidence, and the dotted arrows indicate a proposed relationship between those effects. Shading around multiple boxes denotes relationships between groups of upstream and downstream effects. Progression of effects is depicted from left to right and color coded (grey, exposure; green, initial event; blue, intermediate event; orange, apical event). Here, apical events generally reflect results of epidemiologic studies, which often observe effects at the population level. Epidemiologic evidence may also contribute to upstream boxes. When there are gaps in the evidence, there are complementary gaps in the figure and the accompanying text below.

When considering the available health evidence, there are plausible pathways connecting inhalation of $PM_{2.5}$ to the apical reproductive and developmental events reported in epidemiologic studies (Figure 9-1). The biological plausibility for $PM_{2.5}$-induced effects on reproduction and fertility is supported by evidence from the 2009 PM ISA (U.S. EPA, 2009) and by new evidence. Once these pathways are initiated, there is evidence from experimental and epidemiologic studies that $PM_{2.5}$ inhalation may result in a series of physiological responses that could lead to male and female reproductive effects and altered fertility (e.g., fertility, fecundity, reproduction). The evidence for the initial events (Figure 9-1) that could result in inhalation of $PM_{2.5}$ having on effects fertility and reproduction includes translocation of particles less than 200 nm and/or their soluble components (Chapter 4); and respiratory tract inflammation (Chapter 6). Inhalation of $PM_{2.5}$ can result in translocation of particles or soluble factors from the lungs (see Chapter 5) which then can increase respiratory tract inflammation, which can be followed by systemic inflammation, e.g., C-reactive protein (CRP, see Chapter 5), even increasing CRP during pregnancy (Lee et al., 2011b). Soluble components of $PM_{2.5}$, and poorly soluble particles that are part of the $PM_{2.5}$ fraction and smaller than approximately 200 nm, may translocate into the systemic circulation and contribute to inflammatory or other processes in extrapulmonary compartments. Beyond these events, there is also evidence from experimental and epidemiologic studies demonstrating that exposure to $PM_{2.5}$ could result in a coherent series of physiological responses that provide biological plausibility for the associations reported in epidemiologic

and laboratory animal studies including altered fertility, fecundity and reproduction (Veras et al., 2009), (Legro et al., 2010), (Slama et al., 2013).

As depicted in Figure 9-1, these initial events can give rise to intermediate events including systemic inflammation from epidemiologic evidence of increased CRP during pregnancy (Lee et al., 2011b), animal studies of altered estrous cycle (Veras et al., 2009), altered ovulation (Veras et al., 2009), or decreased ova quality (Veras et al., 2009), erectile dysfunction in epidemiologic studies (Tallon et al., 2017) genetic and epigenetic changes to sperm and other effects on sperm in epidemiologic studies(Hammoud et al., 2009), (Radwan et al., 2015), (Hansen et al., 2010), and laboratory animal studies(Pires et al., 2011).

Laboratory animals provide the biological plausibility for effects on female reproduction with $PM_{2.5}$ inhalation. Briefly, inhalation of $PM_{2.5}$ affects the female and altered estrous cyclicity, ova quality and ovulation. After inhalation of $PM_{2.5}$, there is elongation of the estrous cycle in female rodents that had been exposed to $PM_{2.5}$ for two generations (Veras et al., 2009), which reduced the total number of estrous cycles over a set time period (Veras et al., 2009). In laboratory animals the inhalation of $PM_{2.5}$ also decreased numbers of ovarian follicles at the antral stage with fewer follicles reaching this terminal stage just before ovulation in 2nd generation offspring (Veras et al., 2009). Also, ova quality is decreased (Veras et al., 2009).

Then there are intermediate effects on sperm after $PM_{2.5}$ inhalation, decreasing sperm quality (Hammoud et al., 2009) or motility(Radwan et al., 2015) in epidemiologic studies, or in rodents decreasing the number of sperm (Pires et al., 2011), affecting spermiation (Pires et al., 2011) or induction of genetic and epigenetic changes to sperm of rodents exposed to $PM_{2.5}$ (Yauk et al., 2008). Sertoli cells, which are important for the process of spermatogenesis, are decreased in laboratory animals after prenatal $PM_{2.5}$ exposure (Pires et al., 2011) and testicular weight and volume are decreased with prenatal $PM_{2.5}$ exposure (Pires et al., 2011). Epidemiologic studies show $PM_{2.5}$ exposure is associated with erectile dysfunction (Tallon et al., 2017).

In laboratory animal studies, parental (male and female) inhalation of $PM_{2.5}$ altered fertility and altered fecundity in the 1st (F1) and 2nd generation (F2) offspring after continuous inhalation of $PM_{2.5}$ from preconception (Veras et al., 2009). Inhalation of $PM_{2.5}$ by laboratory animals resulted in increased time required for a successful mating and fertility and pregnancy indices were significantly changed due to $PM_{2.5}$ inhalation (Veras et al., 2009). In these same animals with inhalation of $PM_{2.5}$, there was a significant increase in rate of the post-implantation loss in G1 and G2 animals (Veras et al., 2009). In epidemiologic studies, increased $PM_{2.5}$ exposure in the month prior to conception was associated with reduced fecundability (Slama et al., 2013) and increased $PM_{2.5}$ during ovulation induction was associated with decreased odds of achieving pregnancy by IVF (Legro et al., 2010). Together, these mechanisms provide plausible pathways by which inhalation of $PM_{2.5}$ could progress from the initial events noted above to altered fertility, fecundity, and reproduction. A schematic characterizing the biological plausibility of $PM_{2.5}$ on reproduction and fertility is shown in Figure 9-1.

$PM_{2.5}$ inhalation could lead to reproductive and developmental health effects on male reproduction, female reproduction or fertility following multiple pathways. Pathways leading to effects in female fertility could begin with particle translocation or solubility of particle contents and inflammation, and oxidative stress that may lead to changes along the female reproduction pathway that impact estrus, ova quality, and ovarian follicle formation. Male reproductive outcomes affected by $PM_{2.5}$ exposure and translocation or solubilization of particle contents can involve inflammation or oxidative stress as well as genetic and epigenetic changes that can contribute to impacts on male reproduction including effects on sperm in laboratory animals and epidemiologic studies and erectile dysfunction in humans. Effects on fertility can begin with the initial particle translocation and solubility, oxidative stress and inflammation, with effects on overall fertility including an increase in rate of the post-implantation loss in laboratory animals as well as epidemiologic evidence of reduced fecundability and decreased odds of achieving pregnancy. While experimental studies involving animals contribute most of the evidence of upstream effects, epidemiologic studies found associations between $PM_{2.5}$ exposure and various outcomes. Together, these proposed pathways provide biological plausibility for epidemiologic results of reproductive and developmental health effects and will be used to inform a causality determination, which is discussed later in the chapter (Section 9.1.5).

9.1.1.2 Male Reproduction

Epidemiologic Evidence of Male Reproductive Function

A limited amount of research has been conducted to examine the association between $PM_{2.5}$ and male reproductive outcomes. In the studies of sperm parameters, there is some evidence for decreased motility (Hammoud et al., 2009), including after adjustment for some copollutants (i.e., NO_X, CO) (Radwan et al., 2015), and evidence for association with abnormal morphology is inconsistent, with a study finding higher percent abnormal sperm with higher $PM_{2.5}$ levels (Radwan et al., 2015) and a U.S. study reporting no evidence of associations between $PM_{2.5}$ exposure and sperm morphology (Hansen et al., 2010). Among participants in the National Social Life, Health, and Aging Project (NSHAP), Tallon et al. (2017) observed positive associations between exposure to annual $PM_{2.5}$ concentrations and erectile dysfunction in men aged 57−85 years (OR: 1.26; 95% CI: 0.81, 1.96)[75]. Effect estimates were similar in magnitude and precision when $PM_{2.5}$ concentrations were averaged over 1, 2, 3, 4, 5, 6, or 7 years. In summary, there are some association between $PM_{2.5}$ exposure and some sperm parameters, though the number of studies is limited.

[75] As detailed in the Preface, risk estimates are for a 5 μg/m3 increase in $PM_{2.5}$ concentrations unless otherwise noted.

Toxicological Evidence of Male Reproductive Function

The role of particulate matter exposure on male reproductive function has been explored in a limited number of animal toxicology studies evaluating endpoints including daily sperm production, male reproductive success, male reproductive organ histology and weight or hormonal concentrations and are separated below based on early life PM exposure or adult PM exposure. The results from these studies are summarized in Table 9-1. The 2009 PM ISA (U.S. EPA, 2009) did not include male reproductive studies that are in scope for the current ISA.

In recent work, spermatogenesis was affected in adult animals after prenatal and/or early postnatal exposure of mice to $PM_{2.5}$ (ambient air versus filtered air) from high traffic areas of Sao Paulo, Brazil. Pires et al. (2011) assessed germ cell count, rates of proliferation and apoptosis, spermatid retention and spermatogenic cycle timing. Animals were exposed 24 hour/day for 120 days prior to mating and then throughout pregnancy (prenatal) or for 10 days after birth (postnatal) to ambient or filtered Sao Palo air. Prenatal exposure to ambient air resulted in reduced body weights ($p < 0.001$) and reduced testicular weights ($p = 0.012$) and volume ($p = 0.013$), decreased tubular diameter ($p = 0.004$), and decreased number of elongated spermatids in pre- and postnatal-exposed animals versus filtered air controls. When compared to any other single exposure or the control animals, pre- and postnatal exposure caused significantly higher spermatid head retention at stages VIII–XII, a marker of defective spermiation ($p = 0.004$). No significant changes were detected in Leydig cell, Sertoli cell, spermatogonia, spermatocyte, or round spermatid numbers, or germ cell proliferation, apoptosis, or frequency of spermatogenic stages. The particulate portion of ambient air exposure was responsible for multiple decrements in spermatogenesis in adult animals after early life $PM_{2.5}$ exposure.

Table 9-1 Recent toxicological studies of male reproduction.

Study	Study Population	Exposure Details	Endpoints Examined
(Pires et al., 2011)	Balb/c pregnant mice and male offspring, N = 60, prenatal and postnatal exposure to ambient PM until 90 days of age.	Pregnant dams and male offspring, 120 days (premating through PND 90). $PM_{2.5}$ conc: 16.61 $\mu g/m^3$ nonfiltered air, 2.29 $\mu g/m^3$ filtered air. $PM_{2.5}$ levels were measured gravimetrically by collecting $PM_{2.5}$ particles from cellulose filters obtained using a Harvard impactor.	Effects of pre- and postnatal ambient $PM_{2.5}$ exposure on offspring testis weights, germ cell proliferation, testis morphology, apoptotic germ cells.

In conclusion, mixed effects were seen for associations of $PM_{2.5}$ exposure with male reproductive outcomes. Prenatal and/or early postnatal exposure of mice to $PM_{2.5}$ reduced testicular weight, volume

and tubular diameter, decreased number of elongated spermatids and affected spermiation. Epidemiologic evidence showed positive associations of $PM_{2.5}$ with sperm motility and erectile dysfunction.

9.1.1.3 Female Reproduction

Infertility affects approximately 11% of all women ages 15–44 in the U.S. (Chandra et al., 2013), and can have negative psychological impacts and affect quality of life; infertility and subfertility may also potentially signal poorer physiological health. For example, those with fertility problems are more likely to experience adverse pregnancy and birth outcomes if they do become pregnant (Hansen et al., 2005; Helmerhorst et al., 2004; Jackson et al., 2004). Outcomes evaluated in this section include fecundity, the biologic capacity to reproduce, and fertility, the ability to conceive or induce conception. Researchers may also investigate potential mechanistic links between pregnancy conditions and biomarkers and later birth outcomes; such as pregnancy related hypertension, which is a leading cause of perinatal and maternal mortality and morbidity (Lee et al., 2012b).

Epidemiologic Evidence for Female Reproductive Function

Epidemiologic studies related to fecundity or fertility were not identified for inclusion in the 2009 PM ISA (U.S. EPA, 2009). Recent studies of female reproductive function frequently use populations undergoing assisted reproductive treatment, as these populations have a large amount of data collected on them during treatment and defined menstrual cycles and start points. However, populations undergoing assisted reproductive treatment may be less healthy than the general population of reproductive age. In cohorts recruited from the general population, exact timing can be difficult to determine due to reliance on participant recall, particularly if they are surveyed well after initiation of pregnancy attempts. Many pregnancies are unplanned, which also adds a level of complication to quantifying fertility. Overall, a limited body of evidence provides modest evidence that both short- and long-term $PM_{2.5}$ exposure is associated with decreased fecundability, but did not observe associations between $PM_{2.5}$ exposure and fertility.

Several recent epidemiologic studies examined the association between exposure to air pollutants and the reproductive function or fertility. Gametes (i.e., ova and sperm) may receive higher exposures while outside of the human body, as occurs with assisted reproduction. A recent study estimated daily concentrations of criteria pollutants at addresses of women undergoing their first in vitro fertilization (IVF) cycle and at their IVF labs from 2000 to 2007 in the northeastern U.S. (Legro et al., 2010). Increasing $PM_{2.5}$ concentration estimated at the patient's address during ovulation induction (short-term exposure, ~12 days) was associated with a decreased odds of achieving pregnancy (determined by serum pregnancy test; OR: 0.90; 95% CI: 0.82, 0.99) or an intrauterine pregnancy (determined by ultrasound; OR: 0.90; 95% CI: 0.82, 0.99). These authors observed generally null associations with odds of a live birth after pregnancy was established when $PM_{2.5}$ concentrations were averaged over a number of

exposure periods during pregnancy. The results of this study indicate that short-term $PM_{2.5}$ exposure during ovulation was detrimental and reduced the likelihood of becoming pregnant. Among the general population in the Czech Republic, increased $PM_{2.5}$ exposure in the 30 days before initiation of unprotected intercourse also was associated with reduced fecundability [fecundability ratio: 0.93 (95% CI: 0.88, 0.98), (Slama et al., 2013)].

In an analysis of the Nurses' Health Study II Mahalingaiah et al. (2016), observed null associations with infertility and long-term $PM_{2.5}$ exposure using national spatiotemporal models. They also found no evidence of association with endometriosis, a condition potentially linked to infertility (i.e., attempting to get pregnant for at least one year without success) (Mahalingaiah et al., 2014). Interpolation methods were used to estimate monthly $PM_{2.5}$ concentrations before 1999 in both of these analyses. Of the other recent studies, a cross-sectional study in Spain also reported null associations with fertility rates based on number of live births per 1,000 women aged 15−44 years (Nieuwenhuijsen et al., 2014), while a study of almost 2,000 couples in the Czech Republic found increased $PM_{2.5}$ exposure in the 60 days before initiation of unprotected intercourse was associated with reduced fecundity (Slama et al., 2013). Slama et al. (2013) also examined exposure in the 30 days post-conception as a negative control and observed no evidence of association between $PM_{2.5}$ and fecundity in this period, providing greater certainty for the observed effect of $PM_{2.5}$ exposure on fecundity in their study.

In summary, recent epidemiologic studies showed short-term $PM_{2.5}$ exposure during ovulation was detrimental and reduced the likelihood of becoming pregnant in women undergoing IVF, and in a separate study increased $PM_{2.5}$ exposure in the 30 days before initiation of unprotected intercourse also was associated with reduced fecundability. Little evidence exists in the literature for laboratory animal studies on this outcome. Overall, there appears to be some association between $PM_{2.5}$ exposure and reproductive function (i.e., fecundity outcomes), though the number of studies is limited. In addition, each of these studies account for fertility or fecundity in a different manner, making it difficult to directly compare results across studies. Studies of female reproductive function are summarized in Supplemental Table S9-1 (U.S. EPA, 2018).

Animal Toxicological Evidence for Female Reproduction

Multiple animal toxicological studies of female fertility and estrus from the 2009 PM ISA (U.S. EPA, 2009) reported altered estrous cycles, increased time necessary for mating, smaller litter sizes with increased resorptions and fetal deaths, decreased fertility index, and increased pregnancy index in rodents exposed to $PM_{2.5}$, often ambient air in Sao Paulo, Brazil (Veras et al., 2009). $PM_{2.5}$ inside both chambers and in the outside environment was determined gravimetrically using Harvard impactors.

$PM_{2.5}$ exposure preconception, during gestation or in utero can potentially affect litter size by changing the number of pups conceived or by inducing pup loss during pregnancy or decreasing the number of fertilizations or implantation sites. The 2009 PM ISA (U.S. EPA, 2009) reported significant

changes to litter size with $PM_{2.5}$ exposure. In recent work, litter size was not affected by prenatal exposure of B6C3F1 hybrid mice to Sterling Forest, NY $PM_{2.5}$ CAPs (Klocke et al., 2017) 6 hour each day for most of gestation. Across multiple studies, preconception plus gestational exposure of dams to $PM_{2.5}$ significantly decreased litter size, but paternal exposure plus gestational exposure or gestational exposure alone were not sufficient to affect litter size. More details of these studies are in Table 9-2 below.

Table 9-2 Key toxicological studies of effects of $PM_{2.5}$ on female reproductive function.

Study	Population	Exposure Details	Endpoints Examined
(Klocke et al., 2017)	Male and female B6C3F1 mice (8–10 weeks old) were mated and then dams were exposed to Sterling Forest CAPs.	Prenatal exposure to filtered air or Sterling Forest CAPs for 6 hours/day during gestation (GD0.5 to GD 16.5). Mean CAPs concentration over the exposure period averaged 92.696±19.16 (mean ± SD) μg/m^3 compared to 3.526±0.87 μg/m^3 for FA controls. CAPs exposure levels ranged from 32.95 to 184.43 μg/m^3 over the duration of the exposure period.	Reproductive success.

In conclusion, a recent study exists on animal reproductive success (litter size) with null findings, but no other new studies in the animal toxicology literature on female fertility or estrous cycle have been published since the 2009 PM ISA (U.S. EPA, 2009). The recent epidemiologic literature contains studies on infertility with a U.S. study showing null associations with $PM_{2.5}$ and a Czech study showing positive associations of infertility with $PM_{2.5}$. Epidemiologic associations between $PM_{2.5}$ and endometriosis were null.

9.1.2 Pregnancy and Birth Outcomes

9.1.2.1 Biological Plausibility

This section describes biological pathways that potentially underlie reproductive and developmental health effects of pregnancy, birth weight, and birth outcomes resulting from exposure to $PM_{2.5}$. Figure 9-2 graphically depicts the proposed pathways as a continuum of upstream events, connected by arrows, that may lead to downstream events observed in epidemiologic studies. This discussion of "how" exposure to $PM_{2.5}$ may lead to reproductive and developmental health effects contributes to an understanding of the biological plausibility of epidemiologic results evaluated later in Section 9.1.2.

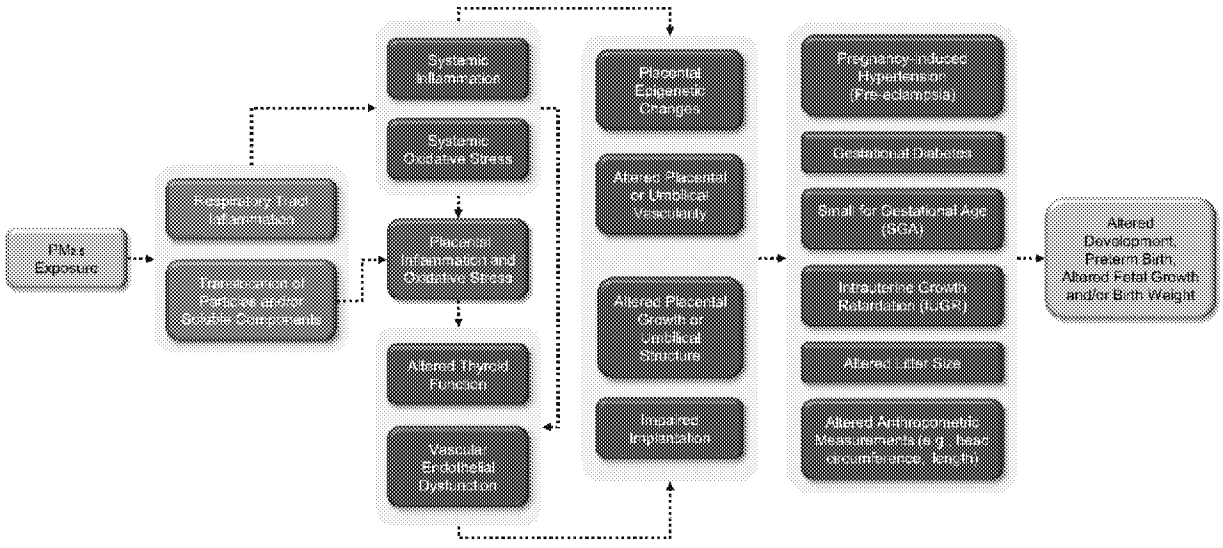


Figure 9-2 Potential biological pathways for pregnancy and birth outcomes following $PM_{2.5}$ exposure

[a] Note: The boxes above represent the effects for which there is experimental or epidemiologic evidence, and the dotted arrows indicate a proposed relationship between those effects. Shading around multiple boxes denotes relationships between groups of upstream and downstream effects. Progression of effects is depicted from left to right and color coded (grey, exposure; green, initial event; blue, intermediate event; orange, apical event). Here, apical events generally reflect results of epidemiologic studies, which often observe effects at the population level. Epidemiologic evidence may also contribute to upstream boxes. When there are gaps in the evidence, there are complementary gaps in the figure and the accompanying text below.

Evidence is accumulating that $PM_{2.5}$ exposure may affect pregnancy and birth outcomes. The evidence from the 2009 PM ISA (U.S. EPA, 2009) and new evidence indicates multiple initial events after $PM_{2.5}$ inhalation contribute to effects on pregnancy and birth outcomes including translocation of particles/soluble components (Valentino et al., 2016); systemic inflammation or oxidative stress. Beyond these initial events, there is also evidence from experimental and epidemiologic studies demonstrating that $PM_{2.5}$ inhalation could result in a coherent series of physiological responses that provide biological plausibility for the associations reported in epidemiologic studies and animal toxicological studies that contribute to the apical endpoint of altered development, preterm birth, altered fetal growth or birth weight. The initial event of systemic oxidative stress is demonstrated in the epidemiologic literature with $PM_{2.5}$-dependent increased odds of elevated c-RP levels during pregnancy (Lee et al., 2011b) or in nonpregnant individuals (Devlin et al., 2014). $PM_{2.5}$-dependent reproductive organ specific inflammation includes placental oxidative stress and intrauterine inflammation (Nachman et al., 2016; Saenen et al., 2016), altered umbilical cord blood lymphocyte distribution (Herr et al., 2010), and increased inflammation along the lipoxygenase pathway in cord blood (5-LOX, 12/15 LOX pathways) (Martens et al., 2017). With increased $PM_{2.5}$ exposure intermediate endpoints emerge with the epidemiologic literature showing altered fetal thyroid function (Janssen et al., 2016; Lavigne et al., 2016a) and altered fetal metabolism (Janssen et al., 2016; Lavigne et al., 2016a). With increased $PM_{2.5}$ exposure, changes to metabolism are seen with increased risk of gestational diabetes (Hu et al., 2015) during the second

trimester. Impaired fetal or maternal thyroid function during a pregnancy can impact the pregnancy, birth outcomes and development. As shown in Figure 9-2, the initial mechanisms can contribute to downstream intermediate effects in laboratory animals including placental or umbilical cord vascularity changes (Veras et al., 2012), endothelial dysfunction (Veras et al., 2012), altered thyroid function (Janssen et al., 2016; Lavigne et al., 2016a) or altered umbilical cord structure (Veras et al., 2012), and in epidemiologic studies of placental genetic or epigenetic changes (Janssen et al., 2013), altered placental growth (Saenen et al., 2015) and impaired implantation (Saenen et al., 2015). One pathway shows impaired placental development including epidemiologic evidence of increased placental inflammation (Saenen et al., 2016), altered expression of placental genes (decreased placental tissue *Bdnf* and *Syn1*) (Saenen et al., 2015), and at the epigenetic level, and human placenta global hypo-methylation with $PM_{2.5}$ exposure (Janssen et al., 2013). Laboratory animal evidence includes altered placental vascularity (Veras et al., 2008), decreased blood vessel diameter on maternal side of placenta and increased capillary surface area on fetal side of placenta (Veras et al., 2008), and decreased placental weight (Veras et al., 2008) (Blum et al., 2017). The line of evidence for effects on the umbilical cord shows $PM_{2.5}$-dependent impairment of the umbilical cord with the epidemiologic literature showing altered cord lymphocyte distribution (Saenen et al., 2016), increased cord blood inflammatory markers (e.g., upregulation of the 5-LOX pathway) (Martens et al., 2017), and laboratory animal evidence of impaired cord artery vascularity (increased endothelin receptor A levels and cord endothelial dysfunction) (Veras et al., 2012), and decreased cord tensile strength (Veras et al., 2012). Decreased fetal growth (Jedrychowski et al., 2010), decreased birth weight (Jedrychowski et al., 2010) and preterm birth (Brauer et al., 2008), (Salihu et al., 2012), (Ha et al., 2014) (Blum et al., 2017) have the strongest evidence in association with $PM_{2.5}$ inhalation and these aforementioned upstream biomarkers provide biological plausibility for these associations. $PM_{2.5}$ exposure has been shown to be associated with pregnancy induced hypertension or pre-eclampsia, gestational diabetes, anthropometric measurements (crown to rump length), IUGR or SGA (Section 9.1.1). There are plausible mechanisms by which inhalation of $PM_{2.5}$ could progress from the initial events noted above to altered growth and development, birth weight, or preterm birth. Supporting evidence is included in Figure 9-2. Together, these proposed pathways provide biological plausibility for epidemiologic results of reproductive and developmental health effects and will be used to inform a causality determination, which is discussed later in the chapter (Section 9.1.5).

In conclusion, decreased fetal growth, decreased birth weight and preterm birth have the strongest evidence in association with $PM_{2.5}$ exposure and these upstream biomarkers provide biological plausibility for these associations. There are plausible mechanisms by which inhalation exposure to $PM_{2.5}$ could progress from the initial events noted above to altered growth and development, birth weight, or preterm birth. Supporting evidence is included in Figure 9-2.

9.1.2.2 Maternal Health during Pregnancy

Epidemiologic Evidence for Effects on Maternal Health during Pregnancy

Studies of maternal health during pregnancy include a number of outcomes, but primarily focus on gestational hypertension disorders and gestational diabetes. Pregnancy-associated hypertension is a leading cause of perinatal and maternal mortality and morbidity. A large body of research has linked changes in blood pressure to ambient air pollution; however, evidence is inconsistent for $PM_{2.5}$ (Section 6.2.6 and Section 6.3.7). A few recent studies have examined whether increases in $PM_{2.5}$ concentrations are associated with hypertensive disorders of pregnancy including preeclampsia (see Supplemental Table S9-1(U.S. EPA, 2018) for study details). The results of these studies were not consistent. The methods by which exposure was assigned in these studies may contribute to the heterogeneity in associations observed across these studies. For example, examination of a cohort from Orange and Los Angeles counties in California revealed that the direction of the association between a composite outcome of gestational hypertensive disorders and $PM_{2.5}$ changed based on how concentrations were determined, either using the CALINE4 model (positive association; OR 1.47; 95% CI: 1.24, 1.68) or the nearest monitor (negative association; OR 0.90; 95% CI: 0.53, 1.54) (Wu et al., 2011; Wu et al., 2009). A cohort study conducted across the U.S. that estimated $PM_{2.5}$ concentrations using a modified CMAQ model across hospital catchment areas reported no evidence of association with preeclampsia for women with or without asthma (Mendola et al., 2016b). A study of around 3,500 women in Washington State observed no associations between preeclampsia and exposure to $PM_{2.5}$ in the seven months following conception when using a LUR exposure model (Rudra et al., 2011). While a larger cohort from Jacksonville, FL, using monitors within 20 km for assignment and with similar average $PM_{2.5}$ concentrations, reported positive odds ratios with any hypertensive disorder and $PM_{2.5}$ exposure in the first and second trimesters (OR: 1.09; 95% CI: 0.99, 1.20; OR: 1.24; 95% CI: 1.11, 1.39, respectively) (Xu et al., 2014). Two meta-analyses have estimated positive odds ratios (ORs 1.15−1.47) for $PM_{2.5}$ and preeclampsia, however both had large heterogeneity scores, and therefore a combined effect may be inappropriate (Hu et al., 2014; Pedersen et al., 2014).

Several studies evaluated the association between short- and long-term $PM_{2.5}$ exposure and gestational hypertension. Two long-term exposure studies of blood pressure report inconsistent effects, with a Pittsburgh study observing null associations (Lee et al., 2012b) and a Polish study reporting positive associations between second trimester $PM_{2.5}$ exposure and blood pressure measured in the third trimester (Jedrychowski et al., 2012). In addition, a study that evaluated short-term $PM_{2.5}$ exposure and blood pressure observed higher blood pressure associated with increased $PM_{2.5}$ in hours 0−4 before delivery in women with gestational hypertension and preeclampsia, but not among normotensive women or women with chronic hypertension (Männistö et al., 2014).

All of the recent studies of gestational diabetes were conducted in areas with average $PM_{2.5}$ concentrations less than 12 $\mu g/m^3$ and provide limited evidence for an association between $PM_{2.5}$ exposure and gestational diabetes. In a nationwide cohort using a specialized CMAQ model and hospital catchment area for exposure, Robledo et al. (2015) reported null associations with $PM_{2.5}$ exposure in the preconception period (OR: 0.97; 95% CI: 0.94, 1.02) and first trimester (OR: 0.98; 95% CI: 0.94, 1.03). In a Florida based study using a hierarchical Bayesian exposure modeling approach, Hu et al. (2015) observed similar results after adjustment for ozone for the first trimester, and also observed increased odds of gestational diabetes with second trimester exposures. These studies were both large, with hundreds of thousands of women in each. In a study of around 2,000 women that compared exposure assignment with monitor values to that with satellite derived concentrations, Fleisch et al. (2014) observed positive associations with impaired glucose tolerance and $PM_{2.5}$ exposure in the second trimester, but null associations with gestational diabetes. In a larger cohort using only satellite derived concentrations Fleisch et al. (2016) again observed no evidence of association between $PM_{2.5}$ in the first or second trimesters and gestational diabetes.

In other outcomes related to pregnancy, $PM_{2.5}$ exposure has been associated with increased odds of high C-reactive protein (Lee et al., 2011b) and altered umbilical cord lymphocyte distributions (Herr et al., 2010), both potentially linked to inflammatory mechanisms for PM, and decreased placental gene expression potentially related to neurodevelopment (Saenen et al., 2015). Recently, $PM_{2.5}$ exposures have also been found to be associated with placental stress measures and intrauterine inflammation (Nachman et al., 2016; Saenen et al., 2016), along with fetal metabolic and fetal thyroid function (Janssen et al., 2016; Lavigne et al., 2016a). Examining short-term $PM_{2.5}$ exposure, Lee et al. (2011b) report elevated ORs for abnormal C-reactive protein levels. The small body of evidence across various pregnancy-related endpoints limits the ability to judge coherence and consistency across these studies, though the positive associations observed in these studies demonstrate that $PM_{2.5}$ exposure could result in physiological responses that contribute to adverse pregnancy outcomes (e.g., preterm birth, altered fetal growth or birth weight).

In summary, there is some evidence for an effect of $PM_{2.5}$ exposure on maternal health during pregnancy. Studies of maternal health during pregnancy are summarized in Supplemental Table S9-1 (U.S. EPA, 2018).

Toxicological Evidence for Effects on Pregnancy

The placenta appears to be a tissue that is sensitive to the downstream effects of $PM_{2.5}$ exposure. The 2009 PM ISA (U.S. EPA, 2009) provided evidence of changes in placental vascularity with $PM_{2.5}$ exposure, including $PM_{2.5}$ dependent decreased placental weight (GD17) with decreased blood vessel diameter on maternal side of placenta and increased capillary surface area on fetal side of placenta (Veras et al., 2008). Recent studies continue to show effects on the placenta in response to $PM_{2.5}$ exposure. Blum et al. (2017) exposed pregnant B6C3F1 hybrid mice to Sterling Forest $PM_{2.5}$ CAPs 6 hours/day and found

that placental weight was significantly decreased with 3rd trimester $PM_{2.5}$ exposure and significantly increased with PM exposure over the entire pregnancy ($p < 0.05$); placental weight was not affected by 1st or 2nd trimester $PM_{2.5}$ exposure. The effect of $PM_{2.5}$ exposure on placental inflammation was followed a 1-hour daily exposure to Sao Palo $PM_{2.5}$ CAPs before and during pregnancy (Blum et al., 2017). Rats were exposed prior to mating and gestational exposure was started at implantation on GD6 and continued through GD19. Animals were exposed for 1 hour/day to CAPs or to HEPA filtered air (de Melo et al., 2015). Placental IL-4 was significantly increased on the fetal side of the placenta ($p < 0.05$) when the dam had combined CAPs exposure before pregnancy and during pregnancy only; none of the other cytokines assessed (IL-1b, IL-4, IL-6, IL-10, INF-g, TNF-a, and Toll-like receptor 4) in both placenta and serum were significantly increased by $PM_{2.5}$ exposure; also, no other exposure paradigms induced significant changes in cytokines. IL-4 protein levels are significantly increased in the fetal portion of the placenta with PM exposure before and during pregnancy, indicating placental inflammation after PM exposure.

More recent work has evaluated the effects of $PM_{2.5}$ on the mouse umbilical cord structural anatomy, microscopic vascular morphology, and markers of oxidative stress (Veras et al., 2012). Dams were exposed to $PM_{2.5}$ (filtered or unfiltered ambient air, Table 9-3 below). The reproductive and developmental outcomes from these animals were reported in previous publications and were covered in the 2009 PM ISA (Veras et al., 2009; Veras et al., 2008). The mean cross-sectional area of umbilical cords from $PM_{2.5}$-exposed group was significantly lower than the filtered air group ($p < 0.001$). The smaller cross-sectional area was due to a significant 28% decrease in total volume of porous mucoid connective tissue (MCT) of the umbilical cord ($p = 0.002$) and the decrease MCT was attributed to a significant 60% loss of collagen in the MCT ($p = 0.002$). PM-exposure resulted in increased oxidative stress or greater levels of immunostaining for 15-F2t-isoprostane in the walls of cord arteries and veins ($p < 0.0001$). Additionally, $PM_{2.5}$ exposure resulted in increased endothelin receptor A levels in cord arteries and veins ($p < 0.0001$), and no changes in endothelin receptor B. Collectively, the results suggest that the reduced birth weights previously reported following particulate exposures may be associated with decreased tensile properties of the umbilical cord due to loss of collagen and with altered blood flow to the fetus.

These studies demonstrate that gestational exposure to $PM_{2.5}$ alters murine umbilical cords and their vessels as well as the placenta, which could potentially deregulate vascular tone, an important contributor to proper fetal development. A summary of the animal toxicological studies of $PM_{2.5}$ exposure is included below in Table 9-3.

Table 9-3 Key toxicological studies of $PM_{2.5}$ exposure and pregnancy and birth outcomes.

Study	Study Population	Exposure Details	Endpoints Examined
(Veras et al., 2012)	BalbC mice (n = 12 dams, per group, fetuses examined in each group). Exposure to ambient air in São. Paulo near high traffic density. Conducted June to November 2006.	Dams were exposed to filtered or unfiltered air (average $PM_{2.5}$ levels, 6.4 µg/m^3 or 32.8 µg/m^3, respectively).	Mouse umbilical cord structural anatomy, microscopic vascular morphology, and markers of oxidative stress.
(de Melo et al., 2015)	Pregnant Female Wistar Rats	Rats were exposed 5 times per week during the 3 weeks before pregnancy and/or 1 time per day each day during pregnancy, starting on GD6 and though GD19. Animals were exposed to $PM_{2.5}$ (ambient $PM_{2.5}$ concentration of 600 mg/m^3 for 1 h). There were 4 exposure paradigms including filtered air (FA) before and during pregnancy (control), PM CAPs before pregnancy +FA during pregnancy, FA before pregnancy + CAPs during pregnancy, or CAPs both before and during pregnancy.	Placental development and systemic inflammation (cytokines, TLR4), pregnant dam blood counts.
(Blum et al., 2017)	Pregnant B6C3F1 hybrid mice, n = 8–17 dams per exposure.	Mice were exposed 6 hours/day to Sterling Forest CAPs during the pregnancy (entire pregnancy or 1st trimester, 2nd trimester, or 3rd trimester). Average daily CAPS concentration ranged from 113 to 192.5 µg/m^3.	Placental weight

9.1.2.3 Fetal Growth, Birth Weight, and Body Length at Birth

Fetal growth can be difficult to quantify; typically, small for-gestational age (SGA) or intrauterine growth restriction (IUGR) are used as dichotomous metrics to characterize suboptimal fetal growth. SGA represents a statistical description of a small neonate, whereas the term IUGR is reserved for those with clinical evidence of abnormal growth. SGA is defined as infants with a birth weight below the 10th percentile for gestational age, usually with consideration for sex and race as well, and is often used interchangeably with IUGR. There are a number of limitations in using SGA/IUGR as a metric of poor fetal growth. One is that a percentile based measure will always quantify a certain percentage of the infant population as growth restricted whether or not this is truly the case (Wollmann, 1998). For example, in term infants, it is unlikely that 10% are actually growth restricted. Whereas in preterm infants, it is likely that more than 10% are growth restricted; therefore, SGA cases would be overestimated in term infants and underestimated in preterm infants. In addition, exact definitions shift between studies and some studies use alternate definitions of SGA/IUGR. For example, some studies use the birth weight distribution of their study population for defining SGA, which will naturally not be identical for every

study population, and others use country standards, which are likely to be more stable, although they may need to be updated with time (Salihu et al., 2012; Brauer et al., 2008).

Birth weight is a measure of fetal growth and an important indicator of future infant and child health. Birth weight is determined by gestational age and intrauterine growth, as well as maternal, placental, fetal and environmental factors. Environmental insults affecting birth weight may occur throughout pregnancy. Implantation or formation of the placenta may be disrupted in the earliest weeks of pregnancy, leading to decreased nutrition throughout pregnancy; or inflammation might result in arterial resistance within the umbilical cord during the later trimesters resulting in poor fetal nutrition. As the largest gains in birth weight occur during the last weeks of gestation, this may be a particularly vulnerable period for birth weight outcomes. Information on birth weight is routinely collected for vital statistics; given that measures of birth weight do not suffer the same uncertainties as gestational age or growth restriction, it is one of the most studied outcomes within air pollution and reproductive health. Birth weight may be examined as a continuous outcome or dichotomous outcome as low birthweight (LBW) (less than 2,500 g or 5 lbs, 8 oz).

There are many methodological issues relating to the study of outdoor air pollution and adverse birth outcomes; and several articles reviewing these methods characterize these challenges (Chen et al., 2010; Woodruff et al., 2009; Ritz and Wilhelm, 2008; Slama et al., 2008). Some of the key challenges to interpretation of birth outcome study results include: the difficulty in assessing exposure as most studies use existing monitoring networks to estimate individual exposure to ambient air pollution; the need for detailed exposure data, and potential residential movement of mothers during pregnancy; the inability to control for potential confounders such as other risk factors that affect birth outcomes (e.g., smoking, correlated air pollutants); evaluating the exposure window (e.g., trimester) of importance; and limited evidence on the physiological modes of action for these effects (Ritz and Wilhelm, 2008; Slama et al., 2008). Some studies have specifically investigated the effects of residential mobility during pregnancy, generally finding movement to similar areas and limited to no effects on PM exposure levels and effect estimates (Pereira et al., 2016; Chen et al., 2010), though a review reported that there may be differences by covariates (Bell and Belanger, 2012). Recently, an international collaboration was formed to better understand the relationships between air pollution and adverse birth outcomes and to examine some of these methodological issues through standardized parallel analyses of data sets across countries (Woodruff et al., 2010) with a study of term birth weight from this collaboration is included in this assessment (Dadvand et al., 2013b). Some of the key challenges to interpretation of these study results include the difficulty in assessing exposure as most studies use existing monitoring networks to estimate individual exposure to ambient PM; the inability to control for potential confounders such as other risk factors that affect birth outcomes; evaluating the exposure window of importance; uncertainty surrounding exposure measurement error, spatial and temporal heterogeneity and limited evidence on the physiological mechanism of these effects. Study of these outcomes can be difficult given the need for detailed data and potential residential movement of mothers during pregnancy. Another uncertainty is whether PM effects differ by the child's sex.

Epidemiologic Evidence for Fetal Growth, Birth Weight, and Body Length at Birth

Studies evaluated in the 2009 PM ISA (U.S. EPA, 2009) generally observed positive associations between $PM_{2.5}$ exposure averaged over the first or second trimester and growth restriction. Among recent studies examining SGA, the evidence is less consistent, with some studies reporting no evidence that increases in $PM_{2.5}$ were associated with increases in odds of SGA (Ha et al., 2017; Stieb et al., 2015; Hannam et al., 2014; Lee et al., 2013), while several others observed that increases in $PM_{2.5}$ were associated with increases in odds of SGA, though magnitude and precision of effects varied (Hyder et al., 2014; Salihu et al., 2012; Rich et al., 2009; Brauer et al., 2008). In the single study of infant anthropometrics and $PM_{2.5}$, small decrements in length and head circumference with log-increases in $PM_{2.5}$ were observed (Jedrychowski et al., 2010).

The 2009 PM ISA (U.S. EPA, 2009) concluded that a limited number of studies conducted in the U.S. observed positive associations between $PM_{2.5}$ exposure and LBW, but that the evidence from studies conducted outside of the U.S. was inconsistent. Many recent studies evaluate the association between $PM_{2.5}$ exposure and birth weight, including studies of LBW and birth weight as a continuous measure. Similar to the results reported in the 2009 PM ISA (U.S. EPA, 2009), when examining the entire body of available literature as a whole, the evidence for an effect of $PM_{2.5}$ on birth weight remains inconsistent. For example, among studies that examine LBW, many report positive associations (i.e., increased odds of LBW) with $PM_{2.5}$ exposure (Ha et al., 2017; Cândido da Silva et al., 2014; Dadvand et al., 2014; Ha et al., 2014; Harris et al., 2014; Hyder et al., 2014; Laurent et al., 2014; Dadvand et al., 2013b; Pedersen et al., 2013; Trasande et al., 2013; Ebisu and Bell, 2012; Salihu et al., 2012; Morello-Frosch et al., 2010). A number also report null or negative effect estimates (Ha et al., 2017; Lavigne et al., 2016b; Brown et al., 2015; Stieb et al., 2015; Fleischer et al., 2014; Fleischer, 2014; Gray et al., 2014; Vinikoor-Imler et al., 2014; Laurent et al., 2013; Madsen et al., 2010; Brauer et al., 2008; Parker and Woodruff, 2008). Similar results are reported for studies that examine change in the continuous measure of birth weight, with some reporting associations between $PM_{2.5}$ exposure and decreases in birth weight (Erickson et al., 2016; Tu et al., 2016; Stieb et al., 2015; Gehring et al., 2014; Hyder et al., 2014; Pedersen et al., 2013; Kloog et al., 2012; Darrow et al., 2011; Gehring et al., 2011; Gray et al., 2011; Gray et al., 2010; Morello-Frosch et al., 2010), and others reporting null associations or showing increases in birth weight (Tu et al., 2016; Fleisch et al., 2015; Lakshmanan et al., 2015; Hannam et al., 2014; Vinikoor-Imler et al., 2014; Laurent et al., 2013; Geer et al., 2012; Darrow et al., 2011; Gehring et al., 2011; Bell et al., 2010; Jedrychowski et al., 2010; Madsen et al., 2010; Slama et al., 2010; Parker and Woodruff, 2008). The entire body of available studies are characterized in Supplemental Table S9-2 (U.S. EPA, 2018).

When evaluating studies of $PM_{2.5}$ exposure and fetal growth or birth weight conducted in North America, where the most consistent associations were observed in the 2009 PM ISA (U.S. EPA, 2009), the results of recent studies are less consistent. There are several studies examining fetal growth and birthweight condected in North America with reported mean $PM_{2.5}$ concentrations less than 12 $\mu g/m^3$ (Table 9-4). For example, Brauer et al. (2008) investigated SGA (defined to the cohort) and LBW using

both inverse distance weighting (IDW) from monitors and LUR exposure metrics in Vancouver. Increases in $PM_{2.5}$ over the whole pregnancy period were associated with increased odds of SGA with both exposure metrics, though confidence intervals were wider with the IDW method (OR IDW = 1.10 [0.90, 1.28], OR LUR = 1.10 [1.00, 1.16]) (Brauer et al., 2008). For LBW, ORs for the different exposure metrics were divergent, with a negative association when using IDW and a positive OR when using LUR to assign exposure, though both sets of CIs were wide (Brauer et al., 2008). Another study set across 24 cities in Canada using LUR methods involving both monitors and satellite data reported near null odds ratios for SGA and LBW with $PM_{2.5}$ across the full pregnancy period in fully adjusted models; mean changes in birth weight were negative with increasing $PM_{2.5}$ in the fully adjusted model (Stieb et al., 2015).

Table 9-4 Epidemiologic studies of $PM_{2.5}$ exposure and effects on fetal growth and birth weight.[a]

Study	Study Population	Exposure Assessment	Mean µg/m^3	Odds Ratio (95% CI)[b]
†Brauer et al. (2008) Vancouver, BC Follow-up: 1999–2002 Birth Cohort Study	70,249 live births in study area with data on residential history	IDW based on ground-monitors (n = 7) assigned to postal codes LUR (R^2 = 0.52), cross-validation revealed poor performance of $PM_{2.5}$ LUR model	IDW: 5.1 LUR: 4.0	Term LBW; entire pregnancy IDW: 0.91 (0.68, 1.25) LUR: 1.10 (0.97, 1.25) SGA; Entire pregnancy IDW: 1.09 (0.91, 1.25) LUR: 1.07 (1.00, 1.10)
†Stieb et al. (2015) Multicity, Canada Follow-up: 1999–2008 Birth Cohort Study	3 million singleton live births; 1.57% term LBW and 8.31% SGA	Hybrid of ground monitors, LUR and remote sensing (satellite images) described in Beckerman et al. (2013)	8.4	Term LBW; entire pregnancy 1.01 (0.94, 1.08) Term BW; entire pregnancy −20.5 (−24.7, −16.4) grams SGA; entire pregnancy 1.04 (1.01, 1.07)
†Salihu et al. (2012) Hillsborough County, FL Follow-up: 2000–2007 Birth Cohort Study	103,961 singleton live births; 6.4% LBW and 8.4% SGA	6-day concentrations from 14 ground monitors; maternal residential ZIP code centroid linked to nearest monitor, based on centroid of ZIP code in which monitor was located; exposure dichotomized at median	Median: 11.28	ORs for exposure above median compared to below median LBW; entire pregnancy 1.07 (1.01, 1.12) Very LBW; entire pregnancy 1.14 (1.01, 1.29) SGA; entire pregnancy 1.06 (1.01, 1.11)
†Ha et al. (2014) Florida, US Follow-up: 2004–2005 Birth Cohort Study	423,719 singleton live births; 2.4% term LBW	HBM CMAQ predictions for 2003–2005 at maternal residence	Entire pregnancy: 9.9 T1: 9.7 T2: 9.9 T3: 10.2	Term LBW Entire pregnancy: 1.04 (0.97, 1.11) T1: 1.01 (0.96, 1.07) T2: 1.07 (1.01, 1.12) T3: 1.01 (0.96, 1.06)

Table 9-4 (Continued): Epidemiologic studies of $PM_{2.5}$ exposure and effects on fetal growth and birth weight.[a]

Study	Study Population	Exposure Assessment	Mean µg/m³	Odds Ratio (95% CI)[b]
†Ha et al. (2017) Multicity, U.S. Follow-up: 2002–2008 Birth Cohort Study	220,572 births, 11.2% SGA; 2.2% term LBW	Population-weighted CMAQ predictions corrected using IDW to local monitors	Entire Pregnancy: 11.8 T1: 11.9 T2: 11.8 T3: 11.9	SGA Entire pregnancy: 1.01 (0.96, 1.07) T1: 1.00 (0.97, 1.04) T2: 1.02 (0.99, 1.06) T3: 1.00 (0.97, 1.03) Term LBW Entire pregnancy: 1.10 (0.97, 1.26) T1: 1.08 (0.99, 1.17) T2: 1.01 (0.93, 1.10) T3: 0.93 (0.86, 1.01)
†Hyder et al. (2014) CT and MA, U.S. Follow-up: 2000–2006 Birth Cohort Study	662,921 births, 2% term LBW, 10% SGA	Weekly averages from closest ground monitors within 50 km of maternal residence Satellite-based predictions from calibration and modeling approach [see (Lee et al., 2012a; Lee et al., 2011a)]	Monitors Entire Pregnancy: 11.9 T1: 12.0 T2: 11.9 T3: 11.8 Satellite (1) Entire Pregnancy: 11.2 T1: 11.2 T2: 11.2 T3: 11.1	Term LBW; entire pregnancy Monitor: 1.02 (0.96, 1.08) Satellite 1: 1.13 (0.94, 1.36) Satellite 2: 1.17 (1.02, 1.36) Term BW; entire pregnancy Monitor: −12.9 (−16.4, −9.5) Satellite 1: −32.6 (−42.5, −22.4) Satellite 2: −93.4 (−47.7, −30.9) SGA; entire pregnancy Monitor: 1.06 (1.02, 1.08) Satellite 1: 1.13 (1.06, 1.22) Satellite 2: 1.17 (1.08, 1.24)
†Kloog et al. (2012) Massachusetts, U.S. Follow-up: 2000–2008 Birth Cohort Study	634,844 singleton live births from MA Birth Registry	Satellite-based predictions from modeling approach [see (Kloog et al., 2011; Lee et al., 2011a)]	9.6	Term BW Entire pregnancy: −4.40 (−5.16, −2.22) 30 days before birth: −4.6 (−7.5, −1.65) 90 days before birth: −7.9 (−10.55, −3.03)
†Lakshmanan et al. (2015) Boston, MA Follow-Up: 2002–2009 Pregnancy Cohort Study	955 singleton births to mothers enrolled in Asthma Coalition on Community, Environment, and Social Stress (ACCESS) cohort	Satellite-based predictions from modeling approach [see (Kloog et al., 2011)] averaged over entire pregnancy	11.0	Birth Weight for Gestational Age (BWGA) z-score; entire pregnancy 0.16 (−0.33, 0.63)

Table 9-4 (Continued): Epidemiologic studies of $PM_{2.5}$ exposure and effects on fetal growth and birth weight.[a]

Study	Study Population	Exposure Assessment	Mean µg/m³	Odds Ratio (95% CI)[b]
†Fleisch et al. (2015) Boston, MA Follow-up: NR Pregnancy Cohort	2,115 singleton live births to mothers enrolled in Project Viva cohort study	Satellite-based predictions from modeling approach [see (Kloog et al., 2011)] averaged over third trimester	11.7	Birth Weight for Gestational Age (BWGA) z-score; third trimester Q1: 1.00 (referent) Q2: −0.02 (−0.14, 0.10) Q3: 0.03 (−0.09, 0.15) Q4: −0.08 (−0.2, 0.04)
†Laurent et al. (2013) Los Angeles, CA 1997–2006 Birth Cohort Study	61,623 term births from network of four hospitals in LA and Orange counties	Ground monitors (closest monitor), CALINE 4 dispersion model; averaged for each month	Monitor: 17.5 CALINE: 4.25	Ground monitor Term LBW Entire pregnancy: 0.93 (0.84, 1.02) birth weight Entire pregnancy: 26.83 (21.56, 32.11) CALINE Term LBW Entire pregnancy: 0.96 (0.74, 1.24) birth weight Entire pregnancy: 21.8 (15.78, 35.18)

[a]This table includes studies conducted in North America in locations where the annual average $PM_{2.5}$ concentration was 20 µg/m³ or less; a complete list of all fetal growth and birth weight studies is included in Supplemental Table S9-2 (U.S. EPA, 2018).

CMAQ = community multiscale air quality modeling system, C-RP = C-reactive protein, EP = entire pregnancy, FR = fecundity ratio M1 = 1st month of pregnancy, IRR = incidence rate ratio, M7 = 7th month of pregnancy, OR = odds ratio, RR = risk or rate ratio, T1 = 1st trimester of pregnancy, T2 = 2nd trimester of pregnancy, T3 = 3rd trimester of pregnancy.

[b]All estimates reported per 5 µg increase in $PM_{2.5}$ unless otherwise stated.

†Studies published since the 2009 Integrated Science Assessment for Particulate Matter.

In the U.S., a Florida study of over 100,000 births using nearest monitor reported $PM_{2.5}$ exposure averaged across the whole pregnancy period to be associated with increased odds of SGA (defined by national standards) and LBW (Salihu et al., 2012). Another Florida cohort study on LBW, using the EPA's Hierarchical Bayesian Prediction Model output for $PM_{2.5}$ and ozone, reported increased ORs with increasing $PM_{2.5}$ exposure for all trimesters after adjustment for ozone (Table 9-4); ORs with the highest magnitude were observed with exposures during the 2nd trimester (Ha et al., 2014). Hyder et al. (2014) investigated associations between $PM_{2.5}$ and fetal growth using exposure assignment for the entire pregnancy period though monitors or through two different satellite models in a Connecticut cohort. They reported increased odds ratios for SGA all methods, though odds ratios from the satellite based methods were of higher magnitude (Hyder et al., 2014). ORs for LBW were elevated for satellite methods, but near null for analyses using monitors, and change in birth weight was negative for all methods, with larger magnitude in satellite analyses (Hyder et al., 2014). Kloog et al. (2012) used a satellite model for $PM_{2.5}$ across the last 30 and 90 days of pregnancy, as well as the full pregnancy period, and observed decreases in birth weight with increasing $PM_{2.5}$ concentrations in Massachusetts. Lakshmanan et al. (2015)

investigated birth weight in a small Boston cohort (n = 670) using modeled air pollution data involving satellite data and LUR across the full pregnancy period. A slightly larger (n = 2,114) study conducted in eastern Massachusetts, also using modeled satellite data for $PM_{2.5}$ exposure in the third trimester, observed an association with lower birth weight only at the highest quartile of exposure (Fleisch et al., 2015). In a southern California study using both monitors and CALINE4 model output (mean $PM_{2.5}$ = 4.25 $\mu g/m^3$), Laurent et al. (2013) report null associations with LBW and increases in birth weight with increases in $PM_{2.5}$ for the entire pregnancy period.

In summary, many recent studies evaluated the relationship between $PM_{2.5}$ exposure and fetal growth and birth weight, and some provide evidence for a positive association for these outcomes. Similar to the results of the 2009 PM ISA (U.S. EPA, 2009), studies in North America generally report detrimental effects on fetal growth with $PM_{2.5\ exposure}$, including a study that adjusted for ozone as a copollutant (Ha et al., 2014). However, recent studies have provided limited evidence to inform uncertainties identified in the last review, including uncertainties related to potential copollutant confounding, the critical window of exposure and plausible biological mechanisms by which $PM_{2.5}$ exposure could result in reduced fetal growth (Section 9.1.2). Studies of fetal growth and birth weight are summarized in Supplemental Table S9-2(U.S. EPA, 2018).

Toxicological Evidence for Fetal Growth, Birth Weight, and Body Length at Birth

Recent studies have examined the effects of $PM_{2.5}$ on fetal growth and birth weight. A summary of these data is included in Table 9-5. The 2009 PM ISA (U.S. EPA, 2009) provided evidence of decreased birth weight with $PM_{2.5}$ exposure during the first week of gestation. Near term C-section birth weight of the pups was significantly decreased when dams were exposed daily to $PM_{2.5}$ (ambient Sao Paulo, Brazil, air for 6 hours/day during the first week of gestation versus filtered air) (Rocha et al., 2008). Multiple recent studies examined effects of PM exposure on birth weight and pup length at birth with mixed findings, possibly due to different exposure windows. Pregnant FVB mice were exposed for 6 hours/day to Columbus, OH, CAPS and bore pups with significantly decreased birthweight ($p = 0.012$) (Gorr et al., 2014). In a separate study, average birth weight and crown-rump length were not affected by prenatal exposure [6 hours/day, of B6CF1 mice to Sterling Forest CAPs for 6 hours/day during most of gestation (Klocke et al., 2017)]. In another study of B6CF1 mice exposed to Sterling Forest CAPs or to filtered air for 6 hours/day had low birth weight associated with PM exposure during the 1st and 2nd trimester or exposure over the entire pregnancy ($p < 0.05$) (Blum et al., 2017). Fetal growth was also assessed in pups collected near term by C-section at GD17 (length, body weight, placental weight) (Blum et al., 2017). Third trimester PM exposure or exposure during the entirety of pregnancy was associated with decrements in fetal growth (weight and body length, [$p < 0.05$]); body length was also significantly decreased with 1st trimester PM exposure ($p < 0.05$). Placental weight was significantly decreased with 3rd trimester PM exposure and significantly increased with PM exposure over the entire pregnancy ($p < 0.05$) (Blum et al., 2017). Birth length was significantly decreased with PM exposure for any period

of PM exposure during pregnancy including 1st, 2nd, or 3rd trimester or the entire pregnancy (Blum et al., 2017). The multiple studies mentioned above assessed birth weight or length in pups after prenatal $PM_{2.5}$ exposure and the majority of these animal toxicology studies show that PM exposure is associated with decreased birth weight of pups or decreased body length at birth (Table 9-5).

Table 9-5 Recent animal toxicological studies of $PM_{2.5}$ exposure and effects on fetal growth and birth weight.

Study	Population N, Sex; Age (mean ± SD)	Exposure Details (Concentration; Duration)	Endpoints Examined
(Blum et al., 2017)	Pregnant B6C3F1 hybrid mice, n = 8–17 dams per exposure.	Mice were exposed 6 h/day to Sterling Forest CAPs during the pregnancy (entire pregnancy or 1st trimester, 2nd trimester, or 3rd trimester). Average daily CAPS concentration ranged from 113 to 192.5 μg/m^3.	Fetal growth at GD17 (body length, body weight)
(Gorr et al., 2014)	Pregnant and lactating FVB mice	Ohio OASIS-1 aerosol concentration system was used to expose dams and pups placed in exposure chambers from GD1 through weaning offspring at 3 weeks. Male offspring at 3 mo of age were then isolated for assessments.	Birth weight
(Klocke et al., 2017)	Male and female B6C3F1 mice (8–10 weeks old) were mated and then dams were exposed to Sterling Forest CAPs.	Prenatal exposure to filtered air or Sterling Forest CAPs for 6 h/day during gestation (GD0.5 to GD 16.5). Mean CAPs concentration over the exposure period averaged 92.696 ± 19.16 (mean ± SD) μg/m^3 compared to 3.526 ± 0.87 μg/m^3 for FA controls.	Birth weight and crown-rump length

Toxicology Evidence for Changes in Anogenital Distance

Measurements of anogenital distance, a marker of androgenization using measurement of the perineum, were collected in pups at PND10 and PND21 (Blum et al., 2017). Pregnant animals were exposed to Sterling forest CAPS for 6 hours/day during one-third of pregnancy or a trimester (1st, 2nd, or 3rd) or during the entirety of pregnancy. In female offspring, significantly decreased AGD was reported with $PM_{2.5}$ exposure in the 1st trimester (PND10 and PND21) and with $PM_{2.5}$ exposure over the entire pregnancy (PND21). Shorter AGD in female rodents is associated with variation in reproductive traits in adulthood (1st estrus, timing of vaginal opening, lordosis) (Zehr et al., 2001). In male pups, AGD mirrored that of female pups at PND21 but not at PND10 (Blum et al., 2017). Both males and females had shortened AGD with 1st trimester CAPs exposure or exposure for the entire pregnancy. AGD length was also sensitive to 2nd trimester in male offspring. The effect of $PM_{2.5}$ exposure in decreasing the AGD is consistent with an anti-androgenic effect of PM exposure on pups.

Toxicological Evidence for Altered Sex Ratio in Litters at Birth

Sex ratio, the ratio of males to females in a litter of animals, is often measured to try to understand if an environmental exposure can contribute to a shift in the ratio of sexes of animals born, an effect that is known to be modulated by stress or other environmental exposures. In a recent study where B6CF1 mice were exposed to Sterling Forest CAPs or to filtered air for 6 hours/day, sex ratio was unaffected by PM exposure at multiple gestational exposure windows (1st, 2nd, or 3rd trimester) and the entirety of pregnancy (Blum et al., 2017).

9.1.2.4 Preterm Birth

Preterm birth (PTB), delivery that occurs before 37 weeks of completed gestation, is a marker for fetal underdevelopment and is related to subsequent adverse health outcomes (e.g., infant mortality, neurodevelopmental problems, growth issues) (Mathews and MacDorman, 2010; Saigal and Doyle, 2008; IOM, 2007; Gilbert et al., 2003). PTB is characterized by multiple etiologies (spontaneous, premature rupture of membranes, or medically induced), and identifying exact causes of PTB is difficult. It is likely that some mechanistic pathways are shared between the three groups; however, isolated causes are also likely to exist. Few, if any, studies distinguish between these three groups in examining associations between air pollution and PTB, though some investigations of premature rupture of membrane (PROM) have been conducted. There is substantial uncertainty surrounding the biological mechanisms leading to PTB, and multiple mechanisms may exist simultaneously.

Epidemiologic Evidence for Preterm Birth and Premature Rupture of Membranes (PROM)

The 2009 PM ISA (U.S. EPA, 2009) included limited number studies evaluating the relationship between $PM_{2.5}$ exposure and PTB, each of which reported a positive association. A number of uncertainties affecting interpretation of the evidence for an association between $PM_{2.5}$ exposure and PTB were identified in the 2009 PM ISA (U.S. EPA, 2009), such as identifying the relevant exposure period. The number of studies evaluating the relationship between $PM_{2.5}$ exposure and PTB has grown considerably in the last decade, and the majority of recent studies report positive associations between $PM_{2.5}$ exposure and PTB, frequently for exposures averaged over the entire pregnancy period (Defranco et al., 2016; Hao et al., 2016; Laurent et al., 2016; Lavigne et al., 2016b; Mendola et al., 2016a; Pereira et al., 2015; Ha et al., 2014; Padula et al., 2014; Pereira et al., 2014a; Chang et al., 2013; Lee et al., 2013; Kloog et al., 2012; Salihu et al., 2012; Warren et al., 2012; Gehring et al., 2011; Wilhelm et al., 2011; Wu et al., 2011; Wu et al., 2009; Brauer et al., 2008). However, while the body of literature has grown considerably since the last review, the evidence from these studies is less consistent than reported in the 2009 PM ISA (U.S. EPA, 2009). Several recent studies report null (Giorgis-Allemand et al., 2017; Mendola et al., 2016a; Hannam et al., 2014; Hyder et al., 2014; Pereira et al., 2014a; Salihu et al., 2012;

Gehring et al., 2011; Rudra et al., 2011; Darrow et al., 2009) or negative (Johnson et al., 2016; Mendola et al., 2016a; Stieb et al., 2015; Pereira et al., 2014a) effect estimates. All of these studies are characterized in Supplemental Table S9-3 (U.S. EPA, 2018).

Many of the studies of $PM_{2.5}$ and preterm birth are conducted in North America, where annual average $PM_{2.5}$ concentrations have decreased considerably in the last decade, and are summarized in Table 9-6. All of the studies included in the 2009 PM ISA (U.S. EPA, 2009) relied on fixed-site monitors to assign exposure $PM_{2.5}$. While many more recent studies have used satellite-based methods or statistical models to assign $PM_{2.5}$ exposure, several recent studies estimated $PM_{2.5}$ concentrations from fixed-site monitors in order to assign exposure. In a study of a cohort from Hillsborough county Florida, Salihu et al. (2012) report ORs elevated from the null with $PM_{2.5}$ exposure using nearest monitor to assign entire pregnancy exposure. In a longitudinal cohort from Rochester NY, which followed 3,264 women over 7,121 pregnancies, positive effect estimates were reported for all trimester exposures, with the highest magnitude with exposures in the first trimester (OR: 1.69, 95% CI: 1.22, 2.29) (Pereira et al., 2015). Effect estimates from this study, which used nearest monitor for exposure assignment, were similar for all buffer distances around monitors (Pereira et al., 2015). Brauer et al. (2008) reported positive ORs using both LUR and IDW in a Vancouver cohort with entire pregnancy exposure (OR: 1.34, 95% CI: 1.05, 1.69). A small Washington state study using LUR to estimate $PM_{2.5}$ exposure over the last 3 months of pregnancy, and a study in New York City utilizing combinations of fixed-site monitoring data and air survey data reported null associations (Johnson et al., 2016; Rudra et al., 2011).

Some recent studies used statistical models or satellite-based methods to estimate exposure to $PM_{2.5}$ when evaluating associations with PTB. In a California-based population, (Wu et al., 2011) observed increased odds of PTB with higher levels of $PM_{2.5}$ estimated with the CALINE 4 dispersion model and averaged over the entire pregnancy period. They also observed higher magnitude effect estimates with very PTB (<30-weeks gestational age) compared to moderate PTB (<35-weeks gestational age) or PTB (<37-weeks gestational age). In a study of a Florida cohort, using the EPA's hierarchical Bayesian CMAQ model output for $PM_{2.5}$ concentrations, Ha et al. (2014) reported positive ORs across all trimesters and for entire pregnancy exposures (entire pregnancy OR: 1.14, 95% CI: 1.10, 1.18). The magnitude of the estimate effects was increased after adjustment for ozone in exposure for first and second trimesters and entire pregnancy (entire pregnancy OR after adjustment for ozone: 1.29, 95% CI: 1.20, 1.38), while those for the third trimester remained positive, but were somewhat attenuated (Ha et al., 2014). Hao et al. (2016) reported a positive association with PTB using fused CMAQ model estimates of $PM_{2.5}$ concentrations in Georgia (U.S.) Lavigne et al. (2016b) and Kloog et al. (2012) observed increased ORs for entire pregnancy exposure to $PM_{2.5}$ estimated with satellite-based models for a cohort of more than 800,000 women in Ontario, Canada and a large Massachusetts cohort, respectively.

Several recent studies evaluated the association between $PM_{2.5}$ exposure and PTB using both fixed-site monitoring data and satellite-based methods to assign exposure. In a cohort set in both Massachusetts and Connecticut, Hyder et al. (2014) reported null associations between PTB and $PM_{2.5}$

exposure over the entire pregnancy period; this study used fixed-site monitors and two separate satellite-based models to estimate exposures; results were consistently null or negative across exposure assignment metrics. Finally, a study of over 2.78 million births across Canada, using a both fixed-site monitor and satellite-based LUR metrics to estimate exposures over the entire pregnancy period, reported inverse ORs with increasing $PM_{2.5}$ exposure (Stieb et al., 2015).

There were no studies included in the 2009 PM ISA (U.S. EPA, 2009) that examined the relationship between $PM_{2.5}$ exposure and PROM. Recent studies evaluate the relationship between both short- and long-term $PM_{2.5}$ exposure and PROM. Effect estimates are inconsistent across recent studies of PROM for long-term $PM_{2.5}$ exposure. An Australian cohort reported elevated ORs with exposure to $PM_{2.5}$ in the second and third trimesters (Pereira et al., 2014b). A U.S. cohort reported relative risks below the null for both PROM and preterm PROM (Wallace et al., 2016), and a small Rochester, NY cohort (n = 3,264) followed over multiple pregnancies reported null associations (Pereira et al., 2015).

Several recent studies examined the association between short-term $PM_{2.5}$ exposure and PTB. Darrow et al. (2009) report null associations using a time-series design with 1-week lagged exposures. Also, using a time-series design, Arroyo et al. (2015) observed positive associations with a 1-day lagged $PM_{2.5}$ exposure, and exposure during week 17 of gestation (Arroyo et al., 2016). Symanski et al. (2014) and Rappazzo et al. (2014) separated PTB into multiple categories based on gestational age. Both observed positive and negative associations depending on combined exposure and outcome period, Symanski et al. (2014) with 4-week exposures, and Rappazzo et al. (2014) with exposures during individual weeks of pregnancy. Warren et al. (2012) also examined exposures at individual weeks of pregnancy, observing elevated associations through week 22 of pregnancy. An additional U.S. study observed positive associations with PROM and $PM_{2.5}$ concentrations estimated from a modified CMAQ model in the 5 hours before hospital admission (Wallace et al., 2016).

In summary, a number of recent studies expand and extend the evidence included in the 2009 PM ISA (U.S. EPA, 2009) for relationship between $PM_{2.5}$ exposure and PTB, though the larger body of literature is somewhat less consistent than the small body of evidence in the 2009 PM ISA. Among studies conducted in North America, where mean $PM_{2.5}$ concentrations tended to be below 12 $\mu g/m^3$, generally positive associations were observed between PTB and $PM_{2.5}$ exposure. This pattern of positive associations was consistent across studies that used fixed-site monitors, statistical models, or satellite-based methods to assign exposure. Addressing an uncertainty identified in the 2009 PM ISA (U.S. EPA, 2009), a study that included a copollutant model including $PM_{2.5}$ and ozone reported the positive association between $PM_{2.5}$ exposure and PTB to be robust to adjustment for ozone. However, timing of exposure, another uncertainty identified in the 2009 PM ISA (U.S. EPA, 2009), varies considerably across these studies and remains an uncertainty in interpreting the results of these studies. In addition to PTB, recent studies also evaluated the relationship between short- and long-term $PM_{2.5}$ exposure and PROM, and outcome that was not included in the 2009 PM ISA (U.S. EPA, 2009). These studies report inconsistent results across studies examining both short- and long-term $PM_{2.5}$ exposures.

Table 9-6 Epidemiologic studies of $PM_{2.5}$ exposure and preterm birth.[a]

Study	Study Population	Exposure Assessment	Mean µg/m³	Effect Estimates 95% CI[b]
Long-term Exposure				
†Wu et al. (2011) LA and Orange Counties, CA, U.S. Follow-up: 2000–2006 Birth Cohort Study	81,186 neonatal records from Memorial Health Care System, a four-hospital network; no birth certificate data used	Nearest monitor (n = 10) Modified CALINE4 line-source dispersion model; focus on local traffic-generated pollution within 3 km of residence at delivery; correlation with measured $PM_{2.5}$ = 0.21	Monitor: 17.3 CALINE: 1.8	Preterm birth (<37 weeks) Monitor, LA, EP: 1.04 (0.94, 1.15) Monitor, Orange, EP: 1.09 (1.00, 1.20) Very preterm birth (<30 weeks) Monitor, LA, EP: 1.03 (0.81, 1.30) Monitor, Orange, EP: 1.33 (0.99, 1.77)
†Brauer et al. (2008) Vancouver, BC Follow-up: 1999–2002 Birth Cohort Study	70,249 live births in study area with data on residential history	Nearest monitor (within 10 km) and IDW (within 50 km) based on ground-monitors (n = 7) assigned to postal codes LUR (R^2 = 0.52), cross-validation revealed moderate performance of $PM_{2.5}$ LUR model (R^2 = 0.52)	Nearest: 5.3 IDW: 5.1 LUR: 4.0	Preterm births (PTB) <37 weeks IDW: EP: 1.34 (1.05, 1.69) Preterm births (PTB) <35 weeks IDW: EP: 1.76 (1.10, 2.93) Preterm births (PTB) <30 weeks IDW: EP: 1.84 (0.66, 5.19)
†Salihu et al. (2012) Hillsborough County, FL Follow-up: 2000–2007 Birth Cohort Study	103,961 singleton live births; 9.1% PTB and 1.1% VPTB	6-day concentrations from 14 ground monitors; maternal residential ZIP code centroid linked to nearest monitor, based on centroid of ZIP code in which monitor was located; exposure dichotomized at median	Median: 11.28	Preterm birth Exposed v. unexposed, EP: 1.03 (0.98, 1.07) Very preterm birth (<33 weeks) Exposed v. unexposed, EP: 1.05 (0.93, 1.18)
†Ha et al. (2014) Florida, US Follow-up: 2004–2005 Birth Cohort Study	423,719 singleton live births; 2.4% term LBW	HBM CMAQ predictions for 2003–2005 at maternal residence	EP: 9.9 T1: 9.7 T2: 9.9 T3: 10.2	Preterm birth T1: 1.06 (1.03, 1.08) T2: 1.25 (1.22, 1.28) T3: 1.05 (1.02, 1.07) EP: 1.14 (1.10, 1.18) Very preterm birth (<32 weeks) T1: 1.12 (1.05, 1.20) T2: 1.45 (1.37, 1.54) T3: 1.02 (0.95, 1.09) EP: 1.22 (1.12, 1.32)

Table 9-6 (Continued): Epidemiologic studies of $PM_{2.5}$ exposure and preterm birth.[a]

Study	Study Population	Exposure Assessment	Mean µg/m³	Effect Estimates 95% CI[b]
†Lavigne et al. (2016b) Ontario, Canada Follow-up: 2005–2012 Birth Cohort Study	N = 818,400	Satellite based model, 1 × 1 km	9.2	Preterm birth EP: 1.10 (1.06, 1.15)
†Hao et al. (2016) Georgia, U.S. Follow-up: 2002–2006 Birth Cohort Study	N = 511,658	Model, fused CMAQ	11.44	Preterm birth EP: 1.05 (1.01, 1.09) T1: 1.00 (0.99, 1.03) T2: 1.03 (1.01, 1.05) T3: 1.01 (0.99, 1.03)
†Pereira et al. (2015) Rochester, NY, U.S. Follow-up: 2004–2012 Birth Cohort Study	N = 3,264 women	Monitor, nearest within 40 km	9	Preterm birth EP: 2.19 (1.40, 3.44) T1: 1.69 (1.22, 2.29) T2: 1.54 (1.10, 2.10) T3: 1.34 (1.00, 1.84)
†Kloog et al. (2012) Massachusetts, US Follow-up: 2000–2008 Birth Cohort Study	634,844 singleton live births from MA Birth Registry	Satellite-based predictions from modeling approach [see (Kloog et al., 2011; Lee et al., 2011a)]	9.6	Preterm birth EP: 1.03 (0.54, 0.63)
†Hyder et al. (2014) CT and MA, US Follow-up: 2000–2006 Birth Cohort Study	662,921 births, 2% term LBW, 10% SGA	Weekly averages from closest ground monitors within 50 km of maternal residence Satellite-based predictions from calibration and modeling approach [see (Lee et al., 2012a; Lee et al., 2011a)]	Monitors EP: 11.9 Satellite (1) EP: 11.4 Satellite (2) EP: 11.2	Preterm birth Monitor: 1.00 (0.98, 1.04) Satellite 1: 0.96 (0.86, 1.04) Satellite 2: 1.00 (0.92, 1.08)
†Rudra et al. (2011) Washington, U.S. Follow-up: 1996–2006 Birth Cohort Study	N = 3,509 women	Land use regression	10.8	Preterm birth Last 3 months: 0.74 (0.39, 1.48)

Table 9-6 (Continued): Epidemiologic studies of $PM_{2.5}$ exposure and preterm birth.[a]

Study	Study Population	Exposure Assessment	Mean µg/m³	Effect Estimates 95% CI[b]
†Johnson et al. (2016) New York City, NY, U.S. Follow-up: 2008–2010 Birth Cohort Study	N = 258,294	Combination of NYC community air survey (spatial) and regulatory monitors (temporal), within 300 m	11	Preterm birth T1: 0.98 (0.95, 1.02) T2: 0.97 (0.94, 1.01) Spontaneous preterm birth T1: 0.99 (0.95, 1.04) T2: 0.99 (0.95, 1.04) Medically indicated preterm birth T1: 0.97 (0.92, 1.03) T2: 0.97 (0.92, 1.04)
†Stieb et al. (2015) Canada 1999–2008 Cohort	N = 2,781,940	Land use regression based on monitor and satellite data to postal code	8.33–8.51	Preterm birth EP: 0.95 (0.92, 0.98)
PROM				
†Pereira et al. (2015) Rochester, NY, U.S. 2004–2012 Longitudinal cohort	N = 3,264 women	Monitor, nearest within 40 km	9	Preterm birth EP: 2.19 (1.40, 3.44) T1: 1.69 (1.22, 2.29) T2: 1.54 (1.10, 2.10) T3: 1.34 (1.00, 1.84) Premature rupture of membranes EP: 1.00 (0.86, 1.22) T1: 0.95 (0.82, 1.10) T2: 0.95 (0.82, 1.16) T3: 0.95 (0.73, 1.22)
†Wallace et al. (2016) U.S. Follow-up: 2002–2008 Birth Cohort Study	N = 223,375	Model, specialized CMAQ, bias corrected with monitor data Averaged over delivery hospital referral region Exposures lagged before hour of admission for delivery	11.9	Preterm premature rupture of membranes Adjusted for all pollutants Lag 0 h: 1.04 (1.00, 1.07) Lag 1 h: 1.04 (1.00, 1.07) Lag 2 h: 1.03 (1.00, 1.07) Lag 3 h: 1.03 (1.00, 1.07) Lag 4 h: 1.03 (1.00, 1.06)
†Pereira et al. (2015) Rochester, NY, U.S. Follow-up: 2004–2012 Birth Cohort Study	N = 3,264 women	Monitor, nearest within 40 km	9	Premature rupture of membranes EP: 1.00 (0.86, 1.22) T1: 0.95 (0.82, 1.10) T2: 0.95 (0.82, 1.16) T3: 0.95 (0.73, 1.22)
Short-term Exposure				

Table 9-6 (Continued): Epidemiologic studies of $PM_{2.5}$ exposure and preterm birth.[a]

Study	Study Population	Exposure Assessment	Mean µg/m³	Effect Estimates 95% CI[b]
†Darrow et al. (2009) Atlanta, GA, U.S. 1994–2004 Time-series	N = 1,994 days, 476,789 births	Monitors, daily population weighted spatial averages from 11 monitors	16.4–16.5	Preterm birth (RR) 1-week lag: 0.98 (0.97, 1.00) Within 4 miles of monitor 1-week lag: 1.00 (0.97, 1.02)
†Symanski et al. (2014) Harris County, Texas, U.S. Follow-up: 2005–2007 Birth Cohort Study	N = 171, 923	Monitors County average	NR	Severe preterm birth (<28 weeks) weeks 1–4: 1.37 (1.15, 1.64) weeks 5–8: 0.95 (0.77, 1.15) weeks 9–12: 1.13 (0.93, 1.37) weeks 13–16: 0.84 (0.70, 1.01) weeks 17–20: 1.30 (1.07, 1.58) Moderately preterm birth (29–32 weeks) weeks 1–4: 1.38 (1.20, 1.59) weeks 5–8: 1.04 (0.88, 1.23) weeks 9–12: 1.28 (1.09, 1.51) weeks 13–16: 0.98 (0.84, 1.15) weeks 17–20: 0.96 (0.82, 1.13) weeks 21–24: 0.94 (0.80, 1.10) weeks 25–28: 1.39 (1.20, 1.61) Mildly preterm birth (33–36 weeks) weeks 1–4: 1.08 (1.02, 1.13) weeks 5–8: 1.04 (0.98, 1.10) weeks 9–12: 1.12 (1.06, 1.05) weeks 13–16: 0.98 (0.93, 1.03) weeks 17–20: 1.08 (1.01, 1.14) weeks 21–24: 0.91 (0.86, 0.96) weeks 25–28: 1.05 (0.99, 1.11) weeks 29–32: 1.14 (1.08, 1.21)
†Rappazzo et al. (2014) Pennsylvania, Ohio, New Jersey, U.S. Follow-up: 2000–2005 Birth Cohort Study	N = 1,940,213	Fused CMAQ model, northeastern U.S. specific Exposures over each week of gestation	14.46	Reported as figures
†Warren et al. (2012) Texas, U.S. Follow-up: 2002–2004 Birth Cohort Study	NR	Monitors CMAQ Exposures over each week of gestation	NR	Reported as figures

Table 9-6 (Continued): Epidemiologic studies of $PM_{2.5}$ exposure and preterm birth.[a]

Study	Study Population	Exposure Assessment	Mean µg/m³	Effect Estimates 95% CI[b]
†Wallace et al. (2016) U.S. Follow-up: 2002–2008 Birth Cohort Study	N = 223,375	Model, specialized CMAQ, bias corrected with monitor data Averaged over delivery hospital referral region Exposures lagged before hour of admission for delivery	11.9	Preterm premature rupture of membranes Adjusted for all pollutants Lag 0 h: 1.04 (1.00, 1.07) Lag 1 h: 1.04 (1.00, 1.07) Lag 2 h: 1.03 (1.00, 1.07) Lag 3 h: 1.03 (1.00, 1.07) Lag 4 h: 1.03 (1.00, 1.06)

[a]This table includes studies conducted in North America in locations where the annual average $PM_{2.5}$ concentration was 20 µg/m³ or less; a complete list of all PTB studies is included in Supplemental Table S9-3 (U.S. EPA, 2018).

CMAQ community multiscale air quality modeling system, C-RP: C-reactive protein, EP: entire pregnancy, FR: fecundity ratio M1: 1st month of pregnancy, IRR: incidence rate ratio, M7: 7th month of pregnancy, OR: odds ratio, RR: risk or rate ratio, T1: 1st trimester of pregnancy, T2: 2nd trimester of pregnancy, T3: 3rd trimester of pregnancy.

[b]All estimates reported per 5 µg increase in $PM_{2.5}$ unless otherwise stated.

†Studies published since the 2009 Integrated Science Assessment for Particulate Matter.

Toxicological Evidence for Preterm birth

The 2009 PM ISA (U.S. EPA, 2009) contained no animal studies of preterm birth. A more recent study monitored pup gestational day at birth to determine if pups were born preterm after CAPs exposure (6 hours/day) during specific windows or trimesters of pregnancy. B6CF1 mouse preterm birth was associated with 2nd, 3rd, or entire pregnancy exposure to Sterling Forest CAPs (Blum et al., 2017). $PM_{2.5}$ exposure during certain periods of pregnancy was associated with preterm birth in mouse pups.

9.1.2.5 Birth Defects

Birth defects are structural and functional abnormalities that can cause physical disability, intellectual disability, and other health problems; they are a leading cause of infant mortality and developmental disability in the U.S. Periods of sensitivity to birth defect development are known for many anomaly types; for example, the critical period of cardiac organogenesis, and thus heart defects, is post-conception weeks 3–8. This knowledge of critical periods means that there are fewer uncertainties around timing of exposure for birth defects compared to other birth outcomes. Birth defects as a category are uncommon, occurring in approximately 3% of live births, and low numbers of specific birth defects can lead to wide confidence intervals in epidemiologic studies investigating environmental causes of birth defects.

Epidemiologic Evidence for Birth Defects

The 2009 PM ISA (U.S. EPA, 2009) synthesized small numbers of studies of PM and birth defects; these often focused on PM10 as the exposure of interest. Though overall numbers remain small, there are several new studies of $PM_{2.5}$ and birth defects, typically cardiac or orofacial defects. These studies are primarily conducted within the U.S., and study populations often arise from states with active birth defect registries, where experts will seek out infants with records of birth defects. One study used data from the National Birth Defects Prevention Study, a large multistate initiative with detailed residential histories and information on many potential confounders, and examined associations between both short- (week long) and longer-term exposure periods (average over post-conception weeks 2–8) and cardiac birth defects (Stingone et al., 2014). In Stingone et al. (2014), median $PM_{2.5}$ levels assigned with monitors across the period of interest were 11.6 $\mu g/m^3$; $PM_{2.5}$ exposure was associated with increased odds of some cardiac defects (hypoplastic left heart syndrome, atrioventricular septal defect), decreased for others (atrial septal defects [ASD]), and null for many. This pattern of results is reflected in the general body of literature for cardiac defects, where several studies have shown either null associations or decreased odds of heart defects (including ASD) with $PM_{2.5}$ exposure (Vinikoor-Imler et al., 2015; Schembari et al., 2014; Agay-Shay et al., 2013; Padula et al., 2013c), while others have reported positive odds ratios (Girguis et al., 2016; Zhang et al., 2016; Salemi et al., 2015; Padula et al., 2013b). Studies of orofacial defects have similar issues, and report inconsistent results (Zhu et al., 2015; Padula et al., 2013a; Marshall et al., 2010). Studies of other types of birth defects have reported positive associations with limb defects (Vinikoor-Imler et al., 2013) and abdominal wall defects (Schembari et al., 2014), and negative associations with sperm disomy (Jurewicz et al., 2014). When examining weekly exposure, Stingone et al. (2014) observed increased odds of Tetralogy of Fallot and pulmonary valve stenosis at higher deciles of $PM_{2.5}$ exposure, and Zhu et al. (2015) observed increased odds of cleft lip with or without cleft palate with $PM_{2.5}$ exposure. In a further analysis of the population analyzed in Stingone et al. (2014), Warren et al. (2016) identified different gestational days as critical $PM_{2.5}$ exposure periods for Tetralogy of Fallot and pulmonary valve stenosis.

In summary, results for most birth defects are inconsistent across studies, or have a limited number of studies, hindering the ability to draw conclusions about this body of literature. Studies of birth defects and $PM_{2.5}$ are characterized in Supplemental Table S9-4 (U.S. EPA, 2018).

Toxicological Evidence for Birth Defects

No previous animal toxicology study addressed birth defects with $PM_{2.5}$ exposure. In a recent study, the effect of $PM_{2.5}$ on exacerbating congenital heart defects was evaluated in an animal model (Chen et al., 2016). Elevated homocysteine levels or hyperhomocysteinaemia during pregnancy, is a risk factor for pregnancy complications including congenital heart defects (Verkleij-Hagoort et al., 2006). $PM_{2.5}$ exposure potentiated the adverse fetal cardiovascular outcomes in rodent pups whose dams were hyperhomocysteinaemic during pregnancy (Chen et al., 2016). In this study, animals were exposed to

ambient $PM_{2.5}$ ($PM_{2.5}$, range 8–68 μg/m^3, mean 36 μg/m^3) in Fuzhou China or filtered air (FA) with particles removed (Chen et al., 2016). Pregnant dams were exposed to $PM_{2.5}$ during pregnancy and lactation and were made hyperhomocysteinaemic at the sensitive window for heart development (G8–G10). Various endpoints including morphological changes to the heart, apoptosis of the myocardium, cardiac progenitor transcriptional factor levels, and cytokine concentrations were studied in the offspring. $PM_{2.5}$ exposure potentiated the adverse morphological changes to the heart (atrial, ventral, or septal heart defects) that were induced by HCY. These morphological changes to the heart were accompanied by changes in myocardial apoptosis, expression of cardiac progenitors (GATA4 and Nkx2–5), and changes in cytokines (TNF-a and IL-1B).

9.1.2.6 Fetal and Infant Mortality

Fetal mortality is the intrauterine death of a fetus. Often these deaths are divided into those occurring before 20 weeks of gestation (spontaneous abortion) and those occurring after (miscarriage/stillbirth). In most areas, fetal deaths are only reported after 20 weeks of completed gestation; this may lead to potential bias, as the population at risk of fetal death is any conception but the actual measured population is only those fetuses reaching at least 20 weeks gestational age. Studies therefore tend to focus on the miscarriage/stillbirth fraction of fetal mortality. Infant mortality is a death occurring in the first year of life, and is divided into two periods: neonatal (i.e., death during the first 28 days), and post-neonatal (i.e., death after the first month of life and before the first birthday). The 2009 PM ISA (U.S. EPA, 2009) reported limited evidence for an association between PM_{10} and fetal mortality (measured as stillbirth) and consistent epidemiologic evidence for an association between PM_{10} exposure and infant mortality, especially due to respiratory causes during the post-neonatal period. A limited number of studies included in the 2009 PM ISA (U.S. EPA, 2009) evaluated the association between $PM_{2.5}$ exposure and infant mortality, and none considered infant mortality due to respiratory causes during the post-neonatal period.

In studies of fetal mortality occurring after 20 weeks of gestation, recent studies generally report positive associations, though timing of exposure varies across studies (Defranco et al., 2015; Green et al., 2015; Faiz et al., 2012). Defranco et al. (2015) reported positive associations with high $PM_{2.5}$ exposure (defined as above mean plus IQR) in entire pregnancy and third trimester, but not first or second trimesters. Green et al. (2015) observed positive associations with entire pregnancy exposures (OR 1.03, 95% CI: 0.99, 1.06), though these associations were attenuated after adjustment for NO_2 (OR 0.98, 95% CI: 0.93, 1.05), and stratification by California air basin resulted in associations with higher magnitudes (e.g., Sacramento Valley OR: 1.16, 95% CI: 1.00, 1.35; San Francisco Bay OR: 1.15, 95% CI: 0.97, 1.36). In a New Jersey study, Faiz et al. (2012) observed positive associations in all trimesters, though slightly stronger ones in the first and second trimesters. In a study of short-term exposures, Faiz et al. (2013) reported a positive association with stillbirth and $PM_{2.5}$ exposure averaged over the two previous days previous, though associations were attenuated to the null after copollutant adjustment (i.e., NO_2,

SO_2). Arroyo et al. (2016) also reported a positive association with short-term $PM_{2.5}$ exposure in gestational week 31 and late fetal death (less than 24 hours after birth). Studies of fetal mortality and $PM_{2.5}$ are characterized in Supplemental Table S9-5 (U.S. EPA, 2018).

The two studies of post-neonatal infant mortality reported positive associations for all-cause mortality, respiratory related mortality, and sudden infant death syndrome (SIDS) (Son et al., 2011b; Woodruff et al., 2008). In the U.S.-based study, the association for respiratory-related mortality (OR: 1.08, 95% CI: 0.97, 1.20) remained positive but was attenuated after adjusting for CO (OR: 1.04, 95% CI: 1.04, 0.92, 1.17), and other gaseous pollutants (i.e., SO_2, and O_3), while the association for SIDS moved away from the null after adjusting for CO in copollutant models Woodruff et al. (2008). In a case-crossover study, Yorifuji et al. (2016) report associations between same day $PM_{2.5}$ and post-neonatal death and all-cause deaths, as well as deaths related to respiratory, SIDS, and birth defects. Studies of infant mortality and $PM_{2.5}$ are characterized in Supplemental Table S9-5 (U.S. EPA, 2018).

9.1.3 Developmental Effects

Pregnancy and infancy are periods of rapid development and exposures occurring during these times may have long-lasting effects that do not manifest immediately (i.e., fetal origins or fetal programming hypothesis). Researchers have examined several health outcomes in associations with exposures during the periods of early development including: cancer (Chapter 8), growth (Chapter 9), infection (Chapter 5), eczema (Chapter 5), neurodevelopmental effects including autism (Chapter 8), cardiovascular effects (Chapter 7) and respiratory effects including asthma (Chapter 5). Of these, respiratory and neurodevelopmental outcomes are the most studied. In addition, these studies of early-life exposure provide evidence that long-term $PM_{2.5}$ exposure is associated with developmental effects (Table 9-7). The developmental studies are characterized in more detail in their respective sections elsewhere in the ISA and are presented here as summaries.

Table 9-7 Summary of developmental effects.

Developmental Effects	Summary of Evidence	Cross-link to Study Details	Causal Determination
Respiratory	Epidemiologic evidence: Studies provide evidence of decrements in lung function growth, asthma development, and respiratory infection.	Section 5.2.2.1 Section 5.2.3.1 Section 5.2.2	**Causal relationship is likely** to exist for long-term exposure to $PM_{2.5}$ and respiratory effects
	Toxicological evidence: Early life exposure to particulate matter has the potential to alter the growth or function of the respiratory system.		
Neurodevelopmental	Epidemiologic evidence: Limited body of evidence does not provide consistent evidence of positive associations with cognitive and behavioral effects or autism.	Section 8.2.7.2	**Causal relationship is likely** to exist for long-term exposure to $PM_{2.5}$ and nervous system effects
	Toxicological evidence: Neurodevelopment in laboratory animal toxicology studies is impacted by $PM_{2.5}$ exposure, including the structural change of ventriculomegaly, and brain inflammatory activation.	Section 8.2.7.2	
Cardiovascular	Epidemiologic evidence: $PM_{2.5}$ exposure was associated with increased odds of some cardiac defects, decreased for others, and null for many.	Section 6.2.5 Section 9.1.2.5	**Causal relationship** exists for long-term exposure to $PM_{2.5}$ and cardiovascular system effects
	Toxicological evidence: Early life exposure to PM in animal models has effects on the developing heart, inducing heart failure in adult animals after early life PM exposure.	Section 6.2.5.2 Section 9.1.2.5	

9.1.3.1 Respiratory Developmental Effects

Epidemiologic Evidence of Respiratory Development

Recent studies evaluate the relationship between $PM_{2.5}$ exposure during the prenatal period and/or the first year of life and respiratory health effects and generally observe positive associations. These studies are characterized in Chapter 5, and include studies of lung development (Section 5.2.2.1), lung function (Section 5.2.2.2.1), asthma development (Section 5.2.3.1) and respiratory infection (Section 5.2.6). Evidence from these studies inform and contribute to the conclusion that there is likely to be a causal relationship between long-term $PM_{2.5}$ exposure and respiratory effects. In addition, these studies of early life exposure provide evidence that long-term $PM_{2.5}$ exposure is associated with developmental effects (Table 9-7).

Toxicological Evidence for Respiratory Development

Early life exposure to particulate matter has the potential to alter the growth or function of the respiratory system. Multiple lines of evidence support that $PM_{2.5}$ or its soluble components can cross the placenta or the maternal fetal barrier to the fetal circulation with the potential to impact the developing fetus (Valentino et al., 2016; Veras et al., 2008). The existing evidence for the current ISA is summarized below in Table 9-7. The 2009 PM ISA (U.S. EPA, 2009) included a study of mice with impaired lung development and lung function after prenatal plus postnatal exposure to ambient $PM_{2.5}$ (Mauad et al., 2008); pulmonary pressure volume analysis demonstrated significant reductions in inspiratory and expiratory volumes and structural aberration included incomplete alveolarization of the lungs. In addition, Pires-Neto et al. (2006) found secretory changes in the nasal cavity of young mice exposed for 5 months to urban $PM_{2.5}$. These findings are discussed in Section 5.2.2.

In studies of DEP and asthma, prenatal DEP exposure increased susceptibility of animals to adult-induced allergic (ovalbumin [OVA]) asthma (significantly increased lung resistance and airway hyper-responsiveness, increased airway inflammation), shifted TH1 and TH2 responses and increased BAL cell counts all in an Aryl Hydrocarbon Receptor (AHR)-dependent mechanism (Manners et al., 2014). Another recent study showed diesel exhaust particulate exposure in utero and allergen exposure in utero conveyed protection from systemic and airway allergic (Aspergillus-induced) immune responses in adult offspring (Corson et al., 2010); adult offspring had a lower immune response when exposed in utero to DE or DE and Aspergillus fumigatus in combination versus allergen.

In another recent study, gestational and early prenatal exposure to Beijing $PM_{2.5}$ is associated with significant lung pathology (peribronchial and perivascular inflammation), increased oxidant production and a decreased antioxidant pool as well as significant changes to circadian clock gene expression (Song et al., 2017). More details on these studies can be found in Section 5.2.2.

9.1.3.2 Neurodevelopmental Effects

Epidemiologic Evidence of Neurodevelopment

Recent studies evaluate the relationship between $PM_{2.5}$ exposure during the prenatal period and/or the first year of life and neurodevelopmental effects and the limited body of evidence does not provide consistent evidence of positive associations. These studies are characterized in Chapter 8, and include studies of cognitive and behavioral effects (Section 8.2.7.1), and autism (Section 8.2.7.2). Evidence from these studies inform and contribute to the conclusion that there is likely to be a causal relationship between long-term $PM_{2.5}$ exposure and nervous system effects. In addition, these studies of early-life exposure provide evidence that long-term $PM_{2.5}$ exposure is associated with developmental effects (Table 9-7).

Toxicological Evidence of Neurodevelopment

The 2009 PM ISA U.S. EPA (2009) contained no studies on neurodevelopmental animal toxicology outcomes. The current ISA explores the effect of $PM_{2.5}$ exposure on behavioral outcomes that can be included in the autism spectrum or as an attention deficit or hyperactivity and structural changes in the brain that may accompany autism, ADHD or mental illness, e.g., ventricular enlargement. A recent study (Klocke et al., 2017) showed that prenatal exposure to CAPs was associated with ventriculomegaly in male and female offspring and increased numbers of activated microglia in the brain as well as multiple other brain structural changes. Females had significantly increased iron deposition in the CC with prenatal CAPs exposure; males had significantly decreased total number of microglia in the CC with a nonsignificant trend trended in this direction for females. Neurodevelopment in laboratory animal toxicology studies is impacted by $PM_{2.5}$ exposure, including the structural change of ventriculomegaly, and brain inflammatory activation. Key details from these studies is summarized in Table 9-7. These studies are discussed in more detail in CHAPTER 8.

9.1.3.3 Cardiovascular Effects

Since the 2009 PM ISA (U.S. EPA, 2009), new studies have evaluated developmental cardiovascular risk in animal models after PM exposure and are described below. The two new studies of cardiovascular effects found PM-dependent heart failure and exacerbation of existing congenital heart defects (birth defects section of the ISA, Section 9.3.1). This new study is summarized in Table 9-7.

Toxicological Evidence of Cardiodevelopment

Work by Gorr et al. (2014) showed prenatal and lactational $PM_{2.5}$ exposure induced heart failure in adult offspring with anatomy (dilated cardiomyopathy with ventricular volume changes, and ventricular wall thickening), functional measures (impaired pressure-volume loops and deficits in contraction length) and cellular manifestation (delayed calcium reuptake during relaxation and reduced response to B-adrenergic stimulation, increased cardiac collagen deposition) confirming heart failure. In work from the same lab, Tanwar et al. (2017) showed that prenatal exposure alone to ambient air PM was sufficient to produce heart failure in adulthood, looking at similar outcomes as Gorr et al. (2014) and mechanisms including acute inflammation in cardiac tissue at birth, and changes in cardiac epigenetic markers (sirtuins and DNA methyltransferases). Early life exposure to PM in animal models has effects on the developing heart, inducing heart failure in adult animals after early life PM exposure. For more details on these studies, see Chapter 6.

9.1.3.4 Postnatal Growth and Development

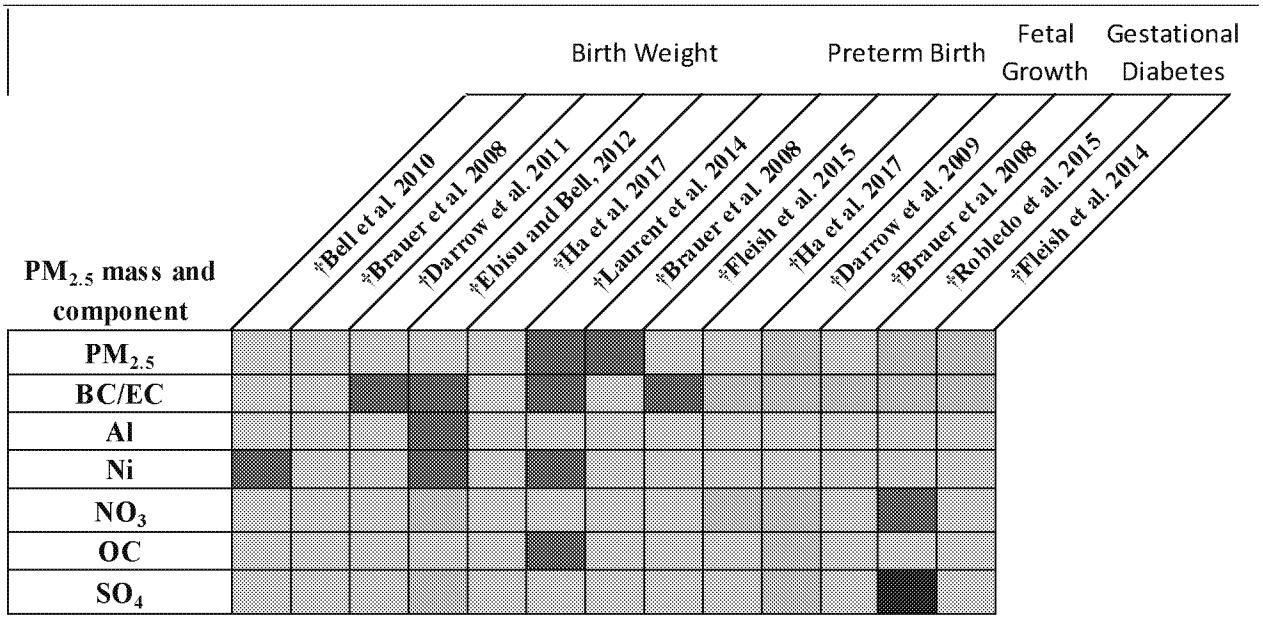
Growth of murine pups in the postnatal period was measured after prenatal exposure to Sterling Forest CAPs. Exposure to CAPs for 6 hours/day during any of the three trimesters of murine pregnancy or during the entire pregnancy was not associated with altered postnatal pup body weight gain in either male or female pups. (Blum et al., 2017).

9.1.4 Associations Between $PM_{2.5}$ Components and Sources and Reproductive and Developmental Effects

In general, few studies have examined associations between $PM_{2.5}$ components and birth outcomes. Elemental carbon (EC) is the component most studied across outcomes, and low birth weight (LBW) is the outcome most commonly evaluated. The evaluation of the association between $PM_{2.5}$ components and reproductive and developmental effects is complicated by the different methods applied across studies. As a result, the systematic standardization of results across studies (i.e., per 5 $\mu g/m^3$ increase), as is the convention throughout this ISA, is not possible when evaluating results for $PM_{2.5}$ components. Overall, the results for individual $PM_{2.5}$ components across studies are generally more imprecise than the results for $PM_{2.5}$ (i.e., much wider confidence intervals, often including the null value), which make the individual results, as well as results across studies, more difficult to interpret. As such, for the purposes of characterizing results with respect to $PM_{2.5}$ components a different convention is employed to evaluate the pattern of associations across studies. Specifically, risk estimates from studies are classified into four categories in Figure 9-3: (1) statistically significant positive associations; (2) positive associations, regardless of width of the confidence interval; (3) null or negative association; and (4) statistically significant negative association. Figure 9-3 demonstrates consistent positive associations for birth weight and preterm birth and exposure to $PM_{2.5}$, BC/EC, OC, and Al, with more studies evaluating $PM_{2.5}$ and BC/EC, and fewer studies examining other components. Based on the pattern of results across this limited number of studies, it is difficult to disentangle the independent effect of any of these components from the effect of $PM_{2.5}$ mass.

Among the studies that examine $PM_{2.5}$ components and LBW, all found positive associations with some components (Ha et al., 2017; Laurent et al., 2014; Ebisu and Bell, 2012; Darrow et al., 2011; Bell et al., 2010). In particular, EC was associated with decrements in birth weight or increased odds of LBW in all studies (Ha et al., 2017; Laurent et al., 2014; Ebisu and Bell, 2012; Darrow et al., 2011; Bell et al., 2010). A four-county cohort in Massachusetts and Connecticut using positive matric factorization to estimate concentrations averaged over the entire pregnancy observed associations with EC, silicon, aluminum, vanadium, and nickel (Bell et al., 2010). Another study included all counties in northeast and mid-Atlantic states with PM composition monitors, reporting positive association between EC, aluminum, calcium, nickel, silicon, titanium, and zinc and LBW or changes in birth weight (Ebisu and Bell, 2012). A study of the five-county Atlanta area reported null associations between $PM_{2.5}$ components and birth

weight in the first month of pregnancy, but both EC and water soluble metals (sum of chromium, copper, iron, manganese, nickel, and vanadium) concentrations were associated with changes in birth weight during the third trimester (Darrow et al., 2011). Laurent et al. (2014), used a spatio-temporal chemical transport model to examine components in Los Angeles county, and observed positive associations between EC, organic carbon, potassium, iron, chromium, nickel, and titanium associated and LBW.



Dark blue = study reported statistically significant positive association; Light blue = study reported a positive association regardless of width of confidence intervals; Light orange = study reported null or negative association; Red = study reported statistically significant negative association; Gray = study did not examine individual component. Only those $PM_{2.5}$ components that were examined in at least three studies are included in this figure.

†$PM_{2.5}$ component studies published since the 2009 PM ISA (U.S. EPA, 2009).

Figure 9-3 Heat map of associations observed between $PM_{2.5}$ and $PM_{2.5}$ components and birth outcomes and effects on pregnancy.

Additional studies have examined the relationship between PM component exposure and fetal growth (Fleisch et al., 2015; Brauer et al., 2008), and preterm birth (Darrow et al., 2009; Brauer et al., 2008). These studies generally report null associations for the components and fetal growth effects.

Among studies of pregnancy, a positive association between gestational diabetes and NO_3 was reported in a large U.S. cohort (Robledo et al., 2015). EC, organic carbon, and ammonium were not associated with gestational diabetes (Robledo et al., 2015; Fleisch et al., 2014).

In summary, there is no evidence than any component(s) is more strongly associated with any reproductive effects than $PM_{2.5}$.

9.1.5 Summary and Causality Determination

Overall, the evidence is suggestive of, but not sufficient to infer, a causal relationship between exposure to $PM_{2.5}$ and (1) male and female fertility and reproduction and (2) pregnancy and birth outcomes. Separate conclusions are made for these groups of reproductive and developmental effects because they are likely to have different etiologies and critical exposure windows over different lifestages. All available evidence examining the relationship between exposure to $PM_{2.5}$ and reproductive and developmental effects was evaluated using the framework described in the Preamble to the ISAs (U.S. EPA, 2015, HEROID). At the time of the 2009 PM ISA (U.S. EPA, 2009), evidence from the epidemiologic and toxicological studies had assessed the broader relationship between $PM_{2.5}$ exposure and reproductive and developmental effects. The 2009 ISA (U.S. EPA, 2009) concluded that the evidence was suggestive for a causal association between PM exposure and reproductive and developmental outcomes. The strongest evidence supporting the causality determination from the 2009 PM ISA (U.S. EPA, 2009) came from studies on low birth weight and developmental outcomes including infant mortality, especially due to respiratory causes during the post-neonatal period. This ISA continues to see strong supporting evidence from low birth weight. There is limited new evidence to inform the relationship between $PM_{2.5}$ and infant mortality from respiratory causes during the post-natal period; developmental outcomes are discussed in more detail in their specific organ system chapter. The developmental animal toxicological evidence has expanded greatly and is characterized elsewhere (respiratory, nervous system). The key evidence, as it relates to the causal framework, is summarized in Table 9-8. **Overall, the evidence is suggestive of, but not sufficient to infer, a causal relationship between $PM_{2.5}$ exposure and (1) Male and Female Reproduction and Fertility, (2) Pregnancy and Birth Outcomes.**

9.1.5.1 Male and Female Fertility and Reproduction

Overall the evidence is suggestive of, but not sufficient to infer a causal relationship between exposure to $PM_{2.5}$ and male and female fertility and reproduction. This is consistent with the 2009 PM ISA, which also concluded the evidence was suggestive of a causal relationship with reproductive and developmental effects. The key evidence supporting the causality determination is detailed below using the framework described in Table I of the Preamble to the ISAs (U.S. EPA, 2015, HERO ID) and is presented in Table 9-8. All available evidence examining the relationship between exposure to $PM_{2.5}$ and pregnancy and birth outcomes was thoroughly evaluated.

The relationship between $PM_{2.5}$ exposure and outcomes related to male and female fertility and reproduction are continuing to be evaluated in the literature, and thus, the number of studies for any one endpoint continues to grow. But questions remain surrounding uncertainties from lack of evaluation of copollutant confounding or multiple potential sensitive windows of exposure. Effects of $PM_{2.5}$ exposure on male reproduction have been studied in both the animal toxicology and the epidemiologic literature.

The strongest effects with $PM_{2.5}$ exposure come from studies on sperm motility (epidemiologic literature) and spermiation (animal toxicology literature). Other studies on sperm including the epidemiologic literature on sperm morphology have inconsistent results. Studies of female reproduction in association with $PM_{2.5}$ exposure also have mixed results. In rodents, ovulation and estrus are affected by PM exposure. In the epidemiologic literature, results on human fertility and fecundity in association with $PM_{2.5}$ exposure is limited, with evidence from IVF showing a modest association of $PM_{2.5}$ concentrations with decreased odds of becoming pregnant. Animal toxicological studies show inconsistent results from $PM_{2.5}$ exposure and its effects on reproduction. Biological plausibility for outcomes on Male and Female Fertility and Reproduction come from laboratory animal studies shown genetic and epigenetic changes to germ cells with $PM_{2.5}$ exposure (Section 9.1.1.1). **Collectively, the evidence is suggestive of, but not sufficient to infer, a causal relationship between $PM_{2.5}$ exposure and male and female reproduction and fertility.**

Table 9-8 Summary of evidence that is suggestive of, but not sufficient to infer, a causal relationship between $PM_{2.5}$ exposure and male and female reproduction and fertility.

Rationale for Causality Determination[a]	Key Evidence[b]	Key References[b]	$PM_{2.5}$ Concentrations Associated with Effects[c]
Limited evidence from multiple epidemiologic studies on sperm quality, fertility and is generally supportive but not entirely consistent	Limited evidence for decreases in sperm motility	Section 9.1.1.2 Hammoud et al. (2009) Radwan et al. (2015)	~15 µg/m^3 34.5 µg/m^3
	Limited evidence for decreased IVF success	Section 9.1.1.3 Legro et al. (2010)	14.08 µg/m^3
	Limited evidence of decreases in fecundability	Section 9.1.1.3 Slama et al. (2013)	34.0 µg/m^3
Limited number of supportive toxicological evidence for effects on male and female fertility and reproduction	Limited evidence for effects on spermatogenesis and spermiation with prenatal or early postnatal exposure	Pires et al. (2011)	16.61 µg/m^3
	Limited evidence of effects on estrous cycle (prolonged cycle), and number of ova (decreased number of antral follicles)	Veras et al. (2009)	27.5 µg/m^3
	Inconsistent evidence of decreased litter size	Veras et al. (2009) (Klocke et al., 2017)	27.5 µg/m^3 92.7 µg/m^3

Table 9-8 (Continued): Summary of evidence that is suggestive of, but not sufficient to infer, a causal relationship between $PM_{2.5}$ exposure and male and female reproduction and fertility.

Rationale for Causality Determination[a]	Key Evidence[b]	Key References[b]	$PM_{2.5}$ Concentrations Associated with Effects[c]
Uncertainty regarding epidemiologic evidence from copollutant models to support an independent $PM_{2.5}$ association	$PM_{2.5}$ effect estimates robust in limited analyses of copollutant models, but generally evaluation of potential copollutant confounding is limited	Radwan et al. (2015)	
Uncertainty due to limited biological plausibility from studies of pregnancy and birth outcomes	Some evidence for initial events that could lead to subsequent effects on sperm, ovulation and the estrous cycle	Section 9.1.1.1 Figure 9-1 Table 9-1	

$PM_{2.5}$ = particulate matter with a nominal aerodynamic diameter less than or equal to 2.5 μm; $PM_{10-2.5}$ = particulate matter with a nominal aerodynamic diameter less than or equal to 10 μm and greater than a nominal diameter of 2.5 μm.

[a]Based on aspects considered in judgments of causality and weight of evidence in causal framework in Tables I and II of the Preamble.

[b]Describes the key evidence and references contributing most heavily to causality determination and, where applicable, to uncertainties or inconsistencies. References to earlier sections indicate where the full body of evidence is described.

[c]Describes the $PM_{10-2.5}$ concentrations with which the evidence is substantiated.

9.1.5.2 Pregnancy and Birth Outcomes

Overall the evidence is suggestive of, but not sufficient to infer a causal relationship between exposure to $PM_{2.5}$ and pregnancy and birth outcomes. This is consistent with the 2009 PM ISA, which also concluded the evidence was suggestive of a causal relationship with reproductive and developmental effects. All available evidence examining the relationship between exposure to $PM_{2.5}$ and pregnancy and birth outcomes was evaluated using the framework described in the Preamble to the ISAs (U.S. EPA, 2015b). The key evidence as it relates to the causal framework is summarized in Table 9-9. There are several well-designed, well-conducted studies that indicate an association between $PM_{2.5}$ and poorer birth outcomes, particularly low birth weight and preterm birth. Albeit, the collective evidence for many of the pregnancy and birth outcomes studies examined is not entirely consistent. There is also evidence for congenital heart defects of different types, as well as biological plausibility to support this outcome from the animal toxicology literature. For preterm birth, the timing of exposure was highly variable from study to study and limited assessment of potential copollutant confounding. The epidemiologic and toxicological literature generally show positive associations of $PM_{2.5}$ exposure with reduced fetal growth and reduced birth weight. Most of the epidemiologic studies do not control for copollutant confounding and do not have a specific sensitive window of exposure, but there is biological plausibility from the

animal toxicological literature in support of these outcomes as well as support for multiple sensitive windows for $PM_{2.5}$ exposure associated outcomes. Various pregnancy related pathologies including gestational hypertension, pre-eclampsia and gestational diabetes show inconsistent results in association with $PM_{2.5}$ exposure. Looking at gestational exposure during the second trimester for gestational diabetes, there are generally positive associations with $PM_{2.5}$ exposure.

There is some information on potential biological plausibility for effects of $PM_{2.5}$ on pregnancy and birth outcomes at relevant exposure levels for this ISA. $PM_{2.5}$ exposure in laboratory rodents induced impaired implantation, induced vascular endothelial dysfunction, and in humans was associated with epigenetic changes to the placenta, and impaired fetal thyroid function (Section 9.1.2.1). All of these pathways have the potential to contribute to the biological plausibility of $PM_{2.5}$ affecting pregnancy and birth outcomes. **In summary, the evidence is suggestive of, but not sufficient to infer, a causal relationship between exposure to $PM_{2.5}$ and pregnancy and birth outcomes.**

Table 9-9 Summary of evidence that is suggestive of, but not sufficient to infer, a causal relationship between $PM_{2.5}$ exposure and pregnancy and birth outcomes.

Rationale for Causality Determination[a]	Key Evidence[b]	Key References[b]	$PM_{2.5}$ Concentrations Associated with Effects[c]
Evidence from multiple epidemiologic studies of fetal growth and birth weight is generally consistent, but uncertainties remain	Positive associations from many studies, but variability in timing of exposure and limited assessment of copollutant confounding	Section 9.1.2 Table 9-6 Table 9-4	Mean concentrations across studies: 4.0–17.5 µg/m^3
Limited toxicological evidence for an effect of $PM_{2.5}$ on fetal growth and birth weight	Limited evidence that $PM_{2.5}$ exposure results in decreased birth weight of pups or decreased body length at birth	Section 9.1.2.3 Table 9-7	
Evidence from multiple epidemiologic studies of preterm birth is generally consistent, but uncertainties remain	Positive associations from many studies, but variability in timing of exposure and limited copollutant models to evaluate potential copollutant confounding	Section 9.1.2.4 Table 9-8 Table 9-4	Mean concentrations across studies: 1.8–22.1 µg/m^3

Table 9-9 (Continued): Summary of evidence that is suggestive of, but not sufficient to infer, a causal relationship between $PM_{2.5}$ exposure and maternal health during pregnancy and birth outcomes.

Rationale for Causality Determination[a]	Key Evidence[b]	Key References[b]	$PM_{2.5}$ Concentrations Associated with Effects[c]
Limited toxicological evidence for an effect of $PM_{2.5}$ on preterm birth	Limited evidence that $PM_{2.5}$ exposure results in preterm birth in mouse pups	Section 9.1.2.4 Blum et al. (2017)	
Limited and inconsistent epidemiologic evidence for other pregnancy and birth outcomes	Some studies observe positive associations between $PM_{2.5}$ and pregnancy, birth defects, and fetal and infant mortality, while other studies observe no consistent pattern of association	Section 9.1.2.2 Section 9.1.2.3 Section 9.1.2.5	
Consistent positive epidemiologic evidence for associations between $PM_{2.5}$ exposure and fetal growth, birth weight and preterm birth across exposure measurement metrics	Positive associations consistently observed across studies that used ground-based (i.e., monitors), model (e.g., CMAQ, dispersion models) and remote sensing (e.g., AOD measurements from satellites) methods, including hybrid methods that combine two or more of these methods.	Table 9-6 Table 9-8	
Uncertainty regarding epidemiologic evidence from copollutant models to support and independent $PM_{2.5}$ association	$PM_{2.5}$ effect estimates robust in limited copollutant models with ozone, but generally evaluation of potential copollutant confounding is limited	Ha et al. (2014)	
Uncertainty due to limited biological plausibility from studies of pregnancy and birth outcomes	Some evidence for initial events that could lead to subsequent altered growth and development or preterm birth	Section 9.1.2.1 Figure 9-2 Table 9-4	

$PM_{2.5}$ = particulate matter with a nominal aerodynamic diameter less than or equal to 2.5 μm.

[a]Based on aspects considered in judgments of causality and weight of evidence in causal framework in Tables I and II of the preamble.

[b]Describes the key evidence and references contributing most heavily to causality determination and, where applicable, to uncertainties or inconsistencies. References to earlier sections indicate where the full body of evidence is described.

[c]Describes the $PM_{2.5}$ concentrations with which the evidence is substantiated.

9.1.5.3 Developmental Outcomes

Developmental outcomes with exposure to $PM_{2.5}$ are summarized in this chapter. Developmental evidence from the 2009 PM ISA (U.S. EPA, 2009) reported $PM_{2.5}$ associated with infant postnatal mortality, with effects stronger in those with respiratory illness. There is recent evidence from both epidemiologic and toxicological studies supporting a relationship between prenatal and childhood $PM_{2.5}$ exposure and effects on postnatal development, including effects on the respiratory, nervous, and cardiovascular systems (Table 9-7). These outcomes, while relevant to the broader reproductive and developmental category, are included in more depth in the specific organ systems of interest where causality determinations are made.

9.2 $PM_{10-2.5}$ Exposure and Reproductive and Developmental Effects

The evidence for effects of $PM_{10-2.5}$ on reproductive and developmental outcomes is characterized below. Infant respiratory mortality and decreased birth weight have the strongest evidence, reporting positive associations. Increased infant respiratory mortality was reported with increasing $PM_{10-2.5}$ exposure. Birth weight is associated with $PM_{10-2.5}$ exposure with reports of decreased birth weight with $PM_{10-2.5}$ exposure and increased odds of having a low birth weight baby with $PM_{10-2.5}$ exposure. Pre-term birth is associated with increasing $PM_{10-2.5}$ exposure as is infertility. Inconsistent evidence is seen with studies of birth defects and studies of pre-term birth with the literature being comprised of studies with positive associations as well as studies with null findings. Male and female reproduction and fertility studies show increased infertility and lower birth rates in epidemiologic studies of $PM_{10-2.5}$. No new studies on effects of $PM_{10-2.5}$ exposure on male and female reproduction and fertility have been reported in the animal toxicology literature. The 2009 PM ISA (U.S. EPA, 2009) contained studies of toxicological effects of $PM_{10-2.5}$ exposure with reproductive effects, but are not within the scope for this ISA. More detailed information on these studies is included in the sections that follow.

9.2.1 Male and Female Reproduction and Fertility

9.2.1.1 Biological Plausibility

There is a paucity of evidence for biological plausibility of health effects following exposure to $PM_{10-2.5}$ due to a dearth of information published in the literature. Thus, a biological plausibility figure

was not constructed for this size fraction. There have been a limited number of studies of reproductive health outcomes focused on $PM_{10-2.5}$ exposure; of these, few examine the same outcome. The studies are reported below as outcomes related to male and female reproduction and fertility.

9.2.1.2 Male and Female Reproduction and Fertility

$PM_{10-2.5}$ exposure has been studied in association with male and female reproduction and fertility in epidemiologic studies and details are reported herein. In examinations of the Nurses' Health Study, authors observed increased incident infertility and reduced endometriosis associated with increased $PM_{10-2.5}$ concentrations from a spatio-temporal model (Mahalingaiah et al., 2016; Mahalingaiah et al., 2014). In a cross-sectional study in Barcelona, Spain Nieuwenhuijsen et al. (2014) reported lower birth rates with increases in $PM_{10-2.5}$ from a land-use regression model.

No new studies on effects of $PM_{10-2.5}$ exposure on male and female reproductive effects and fertility have been reported in the literature. The 2009 PM ISA (U.S. EPA, 2009) contained studies of toxicological effects of $PM_{10-2.5}$ exposure with reproductive effects, but are not within the scope for this ISA.

In conclusion, increased infertility and lower birth rates were reported in epidemiologic studies of $PM_{10-2.5}$. No recent studies of laboratory animals studies on $PM_{10-2.5}$ are reported in this ISA. Overall, there are a limited number of studies which provide inconsistent evidence for an association between $PM_{10-2.5}$ exposure and a variety of reproductive effects. The results of these studies are summarized in Table 9-10.

9.2.2 Pregnancy and Birth Outcomes

9.2.2.1 Biological Plausibility

There is a paucity of evidence for biological plausibility of health effects following exposure to $PM_{10-2.5}$ due to a dearth of information published in the literature. Thus, a biological plausibility figure was not constructed for this size fraction. There have been a limited number of studies of pregnancy and birth outcomes focused on $PM_{10-2.5}$ exposure; of these, few examine the same outcome. The studies are reported below.

9.2.2.2 Pregnancy and Birth Outcomes

Pregnancy and birth outcomes from the epidemiologic literature have been reported in association with $PM_{10-2.5}$exposure and a summary of these studies follows. A Barcelona cohort found positive associations with preeclampsia (Dadvand et al., 2013a). In studies of preterm birth, time-series studies have reported null associations (Darrow et al., 2009) or elevated odds ratios (Salihu et al., 2012). Null effects were observed for PTB in pooled cohort study (ESCAPE) (Giorgis-Allemand et al., 2017). Salihu et al. (2012) observed elevated ORs for low birth weight, and Ebisu et al. (2016) observed small decreases in birth weight with increases in $PM_{10-2.5}$, including with adjustment for $PM_{2.5}$. A study of birth defects found both positive and negative associations with coarse PM exposure (Schembari et al., 2014).

In conclusion, a Barcelona cohort reported positive associations with pre-eclampsia rates, null effects were reported for preterm birth, elevated OR were reported for low birth weight and small decreases in birth weight were all reported in association with increasing $PM_{10-2.5}$. No recent studies of laboratory animals studies on pregnancy and birth outcomes with $PM_{10-2.5}$ exposure are reported in this ISA. Overall, there are a limited number of studies which provide inconsistent evidence for an association between $PM_{10-2.5}$ exposure and a variety of reproductive effects. The results of these studies are summarized in Table 9-10.

Table 9-10 Epidemiologic studies of exposure to $PM_{10-2.5}$ and reproductive effects.

Study	Endpoint Cohort/Location	Mean $PM_{10-2.5}$ $\mu g/m^3$	Exposure Assessment	Single Pollutant Odds Ratio[a] 95% CI	Copollutant Examination
†Mahalingaiah et al. (2014)	Endometriosis (Nurses98 Health Study/14 U.S. States)	10.9	Spatio-temporal models Subtraction method	0.96 (0.91, 1.01)	Correlation (r): NA Copollutant models with: NA
†Mahalingaiah et al. (2016)	Infertility (Nurses' Health Study/14 U.S. States)	11.4	Spatio-temporal models Subtraction method	1.05 (0.99, 1.10)	Correlation (r): NA Copollutant models with: NA
†Dadvand et al. (2013a)	Preeclampsia (Barcelona, Spain)	21.7	LUR model with input from $PM_{10-2.5}$ monitoring campaign	Entire pregnancy: 1.12 (0.84, 1.50) T1: 1.10 (0.79, 1.53) T2: 0.98 (0.74, 1.30) T3: 1.31 (0.96, 1.79)	Correlation (r): NA Copollutant models with: NA

Table 9-10 (Continued): Epidemiologic studies of exposure to $PM_{10-2.5}$ and reproductive effects.

Study	Endpoint Cohort/Location	Mean $PM_{10-2.5}$ $\mu g/m^3$	Exposure Assessment	Single Pollutant Odds Ratio[a] 95% CI	Copollutant Examination
†Darrow et al. (2009)	Preterm birth (Atlanta, GA)	9.1	Single, centrally-located dichot monitor	M1: 1.00 (0.95, 1.04) 1 week before birth: 0.98 (0.95, 1.02) 6 weeks before birth: 1.02 (0.96, 1.08)	Correlation (r): NA Copollutant models with: NA
†Salihu et al. (2012)	Birth weight, fetal growth, preterm birth (Hillsborough County, FL)	13.1	Centroid of ZIP code (n = 97) of residence linked to nearest centroid of ZIP code (n = 14) that included monitors Subtraction method	ORs for exposure >median vs. <median LBW: 1.09 (1.03, 1.15) Very LBW: 1.22 (1.07, 1.39) PTB: 1.05 (1.01, 1.09) Very PTB: 1.13 (1.01, 1.27) SGA: 1.07 (1.02, 1.12)	Correlation (r): NA Copollutant models with: NA
†Giorgis-Allemand et al. (2017)	Preterm birth (13 Cohorts from 11 European countries—ESCAPE cohort)	NR	LUR model with input from $PM_{10-2.5}$ monitoring campaign	Entire pregnancy: 1.00 (0.92, 1.08) T1: 0.99 (0.91, 1.07) T2: 1.00 (0.92, 1.08) Last week: 0.99 (0.94, 1.04) Last month: 0.98 (0.92, 1.02)	Correlation (r): NO_2: 0.71, $PM_{2.5}$: 0.63 Copollutant models with: NA
†Ebisu et al. (2016)	Birth weight (U.S.)	13.7	County-level average from co-located monitors Subtraction method	Change in birth weight (g) Entire pregnancy −4.2 (−4.6, −3.8) T1: −1.3 (−1.7, -0.8) T2: −1.3 (−1.8, −0.9) T3: −1.7 (−2.1, −1.3)	Correlation (r): NA Copollutant models with: $PM_{2.5}$ Entire pregnancy −3.5 (−3.9, −3.0) T1: −1.0 (−1.4, −0.5) T2: −1.2 (−1.6, −0.7) T3: −1.3 (−1.8, −1.0)
†Schembari et al. (2014)	Birth defects (Barcelona, Spain)	21.1	LUR model with input from $PM_{10-2.5}$ monitoring campaign	All cases: 1.01 (0.90, 1.14)	Correlation (r): PM_{10}: 0.89, $PM_{2.5}$: 0.86 Copollutant models with: NA

Table 9-10 (Continued): Epidemiologic studies of exposure to $PM_{10-2.5}$ and reproductive effects.

Study	Endpoint Cohort/Location	Mean $PM_{10-2.5}$ $\mu g/m^3$	Exposure Assessment	Single Pollutant Odds Ratio[a] 95% CI	Copollutant Examination
†Son et al. (2011a)	Infant mortality (Seoul, Korea)	30.6	City-wide average from co-located monitors Subtraction method	All-cause mortality: Entire pregnancy: 1.26 (0.78, 2.04) T1: 0.92 (0.79, 1.07) T2: 0.99 (0.85, 1.15) T3: 1.07 (0.93, 1.22) First year of life: 0.81 (0.67, 0.98) Respiratory mortality: Entire pregnancy: 4.12 (0.69, 24.86) T1: 1.65 (0.99, 2.79) T2: 0.92 (0.54, 1.51) T3: 0.91 (0.57, 1.45) First year of life: 0.41 (0.16, 1.03)	Correlation (r): NA Copollutant models with: NA
†Yorifuji et al. (2016)	Infant mortality (Tokyo, Japan)	$PM_{7-2.5}$: 5.0	Single, centrally-located monitoring station Subtraction method ($PM_{2.5}$ subtracted from suspended particulate matter [SPM; surrogate for PM_{10}])	Infant mortality (all): 0.99 (0.93, 1.05) Infant mortality (CVD): 1.00 (0.79, 1.29) Infant mortality (Resp): 1.24 (0.94, 1.63) Neonatal mortality: 0.88 (0.81, 0.96) Post-neonatal mortality: 1.10 (1.01, 1.19)	Correlation (r): NA Copollutant models with $PM_{2.5}$: Infant mortality (all): 0.97 (0.91, 1.03) Neonatal mortality: 0.87 (0.80, 0.95) Post-neonatal mortality: 1.07 (0.98, 1.17)
†Peel et al. (2011)	Postnatal apnea and bradycardia (Atlanta, GA)	9.6	Single, centrally-located dichot monitor	Apnea: 1.01 (0.99, 1.04) Bradycardia: 1.01 (0.99, 1.02)	Correlation (r): O_3 = 0.40; NO_2 = 0.39; CO = 0.36; SO_2 = 0.19; PM_{10} = 0.76; $PM_{2.5}$ = 0.47 Copollutant models with: NA

[a]Odds Ratio per 5 $\mu g/m^3$ change in $PM_{10-2.5}$ unless otherwise noted.

†Studies published since the 2009 PM ISA (U.S. EPA, 2009).

9.2.3 Developmental Outcomes

Studies of developmental outcomes have been reported from the epidemiologic literature in association with $PM_{10-2.5}$ exposure. Both a study in Seoul, South Korea and a study in Tokyo, Japan found increased infant mortality due to respiratory causes using coarse PM exposure from monitors (Son et al., 2011b) (Yorifuji et al., 2016). For exposures during the postnatal period, Peel et al. (2011) observed no associations between coarse PM and infant apnea and bradycardia.

9.2.4 Summary and Causality Determination

9.2.4.1 Male and Female Fertility and Pregnancy

Overall, the evidence is inadequate to infer the presence or absence of a causal relationship between $PM_{10-2.5}$ exposure and male and female fertility and reproduction. Developmental outcomes are briefly summarized here with causality determination made in the outcome specific chapter (respiratory effects). Separate conclusions are made for the two groups of reproductive and developmental effects because they are likely to have different etiologies and critical exposure patterns over different lifestages. At the time of the 2009 PM ISA (U.S. EPA, 2009), evidence from the epidemiologic and toxicological studies had assessed the broader relationship between PM exposure and reproductive and developmental outcomes. The paucity of evidence for $PM_{10-2.5}$ in the 2009 PM ISA (U.S. EPA, 2009) remains. While there are more recent studies in this ISA, there continue to be fewer studies contributing to this size fraction than to other size groups. Developmental outcomes for the literature are discussed in more detail in the respiratory section of the ISA with infant respiratory mortality having the strongest evidence, reporting positive associations from multiple studies. In the developmental literature increased infant respiratory mortality was reported with increasing $PM_{10-2.5}$ exposure.

Evidence for male and female reproduction and fertility includes work from the Nurses' Health Study which observed increased incident infertility and reduced endometriosis associated with increased $PM_{10-2.5}$ concentrations and cross-sectional work from a Spanish cohort reporting lower birth rates with increases in $PM_{10-2.5}$. There is a dearth of evidence detailing biological plausibility between $PM_{10-2.5}$ and Male and Female Reproduction and Fertility. **Overall, the evidence is inadequate to infer the presence or absence of a causal relationship between $PM_{10-2.5}$ exposure and male and female reproduction and fertility (Table 9-11).**

Table 9-11 Summary of evidence that it is inadequate to infer the presence or absence of a causal relationship between $PM_{10-2.5}$ exposure and male and female reproduction and fertility.

Rationale for Causality Determination[a]	Key Evidence[b]	Key References[b]	$PM_{10-2.5}$ Concentrations Associated with Effects[c]
Limited and inconsistent epidemiologic evidence from on fertility and reproduction	Limited and inconsistent evidence for effects on incident infertility and decreased birth rates	Mahalingaiah et al. (2016) Nieuwenhuijsen et al. (2014)	9.9 µg/m^3 21.6 µg/m^3
Uncertainty regarding exposure measurement error in epidemiologic studies	Across studies, $PM_{10-2.5}$ concentrations are measured using a number of approaches (i.e., directly measured from dichotomous sampler, difference between PM_{10} and $PM_{2.5}$ concentrations measured at collocated monitors, and difference of area-wide concentrations of PM_{10} and $PM_{2.5}$), which have not been compared in terms of whether they have similar spatial and temporal correlations	Section 3.3.1.1	
Uncertainty regarding epidemiologic evidence from copollutant models to support and independent $PM_{10-2.5}$ association	$PM_{10-2.5}$ effect estimate robust to adjustment for $PM_{2.5}$ in a single study. No studies evaluated potential copollutant confounding for gaseous pollutants	Ebisu et al. (2016)	13.7 µg/m^3
Uncertainty due to limited biological plausibility from studies of pregnancy and birth outcomes	Some evidence for initial events that could lead to subsequent effects on sperm, ovulation and the estrous cycle	Section 9.2.1.1 Figure 9-3 Table 9-10	

$PM_{2.5}$ = particulate matter with a nominal aerodynamic diameter less than or equal to 2.5 µm; $PM_{10-2.5}$ = particulate matter with a nominal aerodynamic diameter less than or equal to 10 µm and greater than a nominal diameter of 2.5 µm.

[a]Based on aspects considered in judgments of causality and weight of evidence in causal framework in Tables I and II of the Preamble.

[b]Describes the key evidence and references contributing most heavily to causality determination and, where applicable, to uncertainties or inconsistencies. References to earlier sections indicate where the full body of evidence is described.

[c]Describes the $PM_{10-2.5}$ concentrations with which the evidence is substantiated.

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9.2.4.2 Pregnancy and Birth Outcomes

Overall, the evidence is inadequate to infer the presence or absence of a causal relationship between $PM_{10-2.5}$ exposure and pregnancy and birth outcomes. At the time of the 2009 PM ISA (U.S. EPA, 2009), evidence from the epidemiologic and toxicological studies had assessed the broader relationship between PM exposure and reproductive and developmental outcomes. The paucity of evidence for $PM_{10-2.5}$ in the 2009 PM ISA (U.S. EPA, 2009) remains.

Evidence for pregnancy and birth outcomes in association with $PM_{10-2.5}$ follows. Decreased birth weight is associated with $PM_{10-2.5}$ exposure including increased odds of having a low birth weight baby with $PM_{10-2.5}$ exposure. Preterm birth is associated with increasing $PM_{10-2.5}$ exposure. Inconsistent evidence is seen with studies of birth defects and studies of preterm birth with the literature being comprised of studies with positive associations as well as studies with null findings. A paucity of information exists in support of potential biological plausibility for $PM_{10-2.5}$ exposure and Pregnancy and Birth Outcomes. **Overall, the evidence is inadequate to infer the presence or absence of a causal relationship between $PM_{10-2.5}$ exposure and pregnancy and birth outcomes (Table 9-12).**

Table 9-12 Summary of evidence that it is inadequate to infer the presence or absence of a causal relationship between $PM_{10-2.5}$ exposure and pregnancy and birth outcomes.

Rationale for Causality Determination[a]	Key Evidence[b]	Key References[b]	$PM_{10-2.5}$ Concentrations Associated with Effects[c]
Limited and inconsistent epidemiologic evidence for associations with pregnancy and birth outcomes	Limited and inconsistent evidence for effects on pre-eclampsia, preterm birth, birth weight, birth defects, and infant mortality	Section 9.2.2.1	
Uncertainty regarding exposure measurement error in epidemiologic studies	Across studies, $PM_{10-2.5}$ concentrations are measured using a number of approaches (i.e., directly measured from dichotomous sampler, difference between PM_{10} and $PM_{2.5}$ concentrations measured at collocated monitors, and difference of area-wide concentrations of PM_{10} and $PM_{2.5}$), which have not been compared in terms of whether they have similar spatial and temporal correlations		

Table 9-12 (Continued): Summary of evidence that it is inadequate to infer the presence or absence of a causal relationship between $PM_{10-2.5}$ exposure and pregnancy and birth outcomes.

Rationale for Causality Determination[a]	Key Evidence[b]	Key References[b]	$PM_{10-2.5}$ Concentrations Associated with Effects[c]
Uncertainty regarding epidemiologic evidence from copollutant models to support and independent $PM_{10-2.5}$ association	$PM_{10-2.5}$ effect estimate robust to adjustment for $PM_{2.5}$ in a single study. No studies evaluated potential copollutant confounding for gaseous pollutants	Ebisu et al. (2016)	13.7 $\mu g/m^3$
Uncertainty due to limited biological plausibility from studies of pregnancy and birth outcomes	Some evidence for initial events that could lead to subsequent effects on pregnancy and birth outcomes.	Section 9.2.2.2	

$PM_{2.5}$ = particulate matter with a nominal aerodynamic diameter less than or equal to 2.5 µm; $PM_{10-2.5}$ = particulate matter with a nominal aerodynamic diameter less than or equal to 10 µm and greater than a nominal diameter of 2.5 µm.

[a]Based on aspects considered in judgments of causality and weight of evidence in causal framework in Tables I and II of the Preamble.

[b]Describes the key evidence and references contributing most heavily to causality determination and, where applicable, to uncertainties or inconsistencies. References to earlier sections indicate where the full body of evidence is described.

[c]Describes the $PM_{10-2.5}$ concentrations with which the evidence is substantiated.

9.3 UFP Exposure and Reproductive and Developmental Effects

The evidence for effects of UFP on reproductive and developmental outcomes is characterized below. Toxicological studies of male reproductive function show increased testosterone, increased testicular cholesterol, and increased activation of biomarkers on testicular cholesterol biosynthesis pathway with UFP exposure in male rodents. The epidemiologic literature for pregnancy and birth outcomes shows positive associations of UFP with preterm birth and low birth weight. In the UFP toxicological literature, neurodevelopmental outcomes are well studied and report neurological associations from multiple studies evaluating outcomes including increased impulsivity, ventriculomegaly, glial activation, and neurotransmitter changes with UFP exposure. More detailed information on these studies is included in the sections that follow.

9.3.1 Male and Female Reproduction and Fertility

9.3.1.1 Biological Plausibility

This section describes biological pathways that potentially underlie reproductive and developmental health effects of male and female reproduction and fertility, and pregnancy, birth weight and birth outcomes resulting from exposure to UFP PM. Figure 9-4 graphically depicts the proposed pathways as a continuum of upstream events, connected by arrows, that may lead to downstream events observed in epidemiologic studies. This discussion of "how" exposure to UFP may lead to reproductive and developmental health effects contributes to an understanding of the biological plausibility of epidemiologic results evaluated later in Section 9.3.

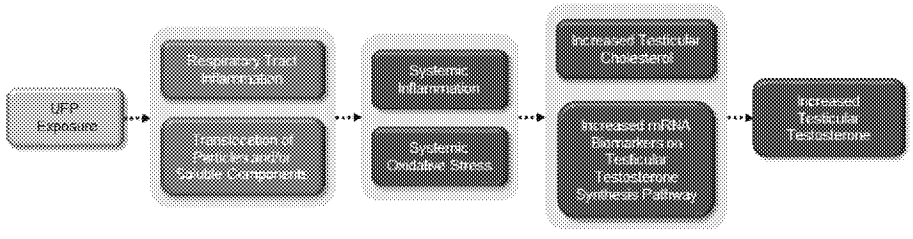

Figure 9-4 Potential biological pathways for male and female reproduction and fertility effects following UFP exposure.

[a] Note: The boxes above represent the effects for which there is experimental or epidemiologic evidence, and the dotted arrows indicate a proposed relationship between those effects. Shading around multiple boxes denotes relationships between groups of upstream and downstream effects. Progression of effects is depicted from left to right and color coded (grey, exposure; green, initial event; blue, intermediate event; orange, apical event). Here, apical events generally reflect results of epidemiologic studies, which often observe effects at the population level. Epidemiologic evidence may also contribute to upstream boxes. When there are gaps in the evidence, there are complementary gaps in the figure and the accompanying text below.